

Aus dem Institut für Tierernährung

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Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier

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Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier



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10. Symposium „Vitamine und Zusatzstoffe“ - Anmerkungen zum Jubiläum

Gerhard Flachowsky: Institut für Tierernährung der Bundesforschungsanstalt für Landwirtschaft (FAL), Bundesallee 50, 38116 Braunschweig

Als am 05.12.1983 Arno Hennig das erste Mal Wissenschaftler und Anwender nach Leipzig rief, um über die Thematik Vitamine zu diskutieren, ahnte wohl kaum einer der Teilnehmer, was in den nächsten Jahren alles geschehen wird und dass im Jahre 2005 dieses Symposium immer noch Bestand haben wird.

In der Begrüßung zu dieser Tagung stellte Hans-Joachim Schwark (Direktor der Sektion Tierproduktion und Veterinärmedizin der Universität Leipzig) fest, dass „...vor 60 Jahren Arthur Scheunert hier ein Zentrum für Vitaminwissenschaft aufzubauen begann, das in Europa eine Spitzenstellung besaß. Scheunert war es auch, der keine Grenzen zwischen Human- und Veterinärmedizin sowie Tierernährung zog, sondern in seine Pionierarbeit Mensch und Tier einbezog.... Es freut mich daher, dass wir hier in Leipzig die alten Traditionen fortsetzen, wobei es dem Geist Scheunerts entspricht, wenn wir die Forscher auf den Gebieten der Humanmedizin, Veterinärmedizin und Ernährungswissenschaft in einem Symposium vereint sehen“.

Aus heutiger Sicht können neben den oben erwähnten Zielen eines gemeinsamen Forums von Human- und Tierernährern bzw. -medizinern als Gründe für die Etablierung der Tagung folgende Aspekte angeführt werden:

- Entwicklung eines internationalen Forums zum Ergebnis-/Gedankenaustausch auf dem Fachgebiet („wenn wir nicht raus können, müssen wir Fachkollegen zu uns holen“)
- Forum zwischen Wissenschaftlern verschiedener Fachdisziplinen, Zusatzstoffherstellern, Herstellern von Mischfutter und Nahrungsergänzungsmitteln, Praktiker u.a.
- Podium für Nachwuchswissenschaftler zur Präsentation ihrer Ergebnisse
- Weitere Profilierung des Standortes Leipzig/Jena mit dem Forschungsschwerpunkt „Vitamine, sonstige Zusatzstoffe sowie Mengen- und Spurenelemente (eine entsprechende Jahrestagung gab es bereits seit 1980).

Abgesehen vom ersten Punkt der vorangestellten Auflistung - jetzt können wir „raus“ - ist die Veranstaltung im Laufe der Jahre diesen Ansprüchen treu geblieben. Das Forum für Human- und Tierernährer, aber auch für Anwender, wurde erhalten, wie die Vielzahl der Beiträge aus beiden Fachgebieten belegen (Tab. 1). Nachwuchswissenschaftler waren und sind immer gern gesehene Referenten, wie aus der Vielzahl der Beiträge, auch des vorliegenden Tagungsbandes, zu entnehmen ist.

Bevor das Symposium im Jahre 1993 - nach der Etablierung des Studienganges Ernährungswissenschaften an der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller Universität - in Jena seine endgültige Heimstätte fand, hatte es eine bewegte Geschichte.

Auf dem 2. Symposium, das im Jahre 1987 in Reinhardsbrunn im Thüringer Wald durchgeführt wurde, äußerte sich Arno Hennig bei der Begrüßung der Teilnehmer zur

Problematik Ergotropika, die in den Titel der Veranstaltung aufgenommen wurden, wie folgt:

„...Wir wissen natürlich, dass die gezielte Einflussnahme von außen zugeführter Substanzen nicht die Spur eines Risikos für den Verbraucher mit sich bringen darf. Bei im Körper gebildeten Substanzen, die ja auch metabolisiert und exkretiert werden können, ist dieses Risiko wesentlich geringer als bei körperfremden...“.

Dieses Statement aus der Zeit des noch prosperierenden Einsatzes von Antibiotika als Futterzusatzstoff zeugt von großer Weitsicht und könnte auch aus der Phase des forcierten Verbraucherschutzes stammen.

Die Folgen der politischen Wende in der DDR stellten für den in Frage stehenden Agrar- und Ernährungsforschungs-Standort Jena auch das Symposium in Frage. Dank der Unterstützung verschiedener Vertreter der Biologischen Fakultät der Friedrich-Schiller-Universität Jena, vor allem des Dekans Eberhard Müller, und des Zuspuches und der Hilfe von außen gelang es, im Jahre 1991 das 3. Symposium in Stadtroda bei Jena zu organisieren.

Wolfram Braune, der Prodekan der Biologischen Fakultät, führte damals in seiner Begrüßung aus „...Wie ich gehört habe, fühlten sich die Organisatoren dieser Tagung längere Zeit etwas unsicher in der Frage, ob dieser Zeitpunkt, zu dem diese Veranstaltung vorgesehen war, und der Ort richtig gewählt sei, und ob überhaupt die gegenwärtigen Bedingungen hier eine solche Unternehmung erlauben würden. Und was sich in solchen Fällen wohl immer bewährt, hat die Entscheidung dann schließlich leichter gemacht: Lieber etwas tun und Risiken in Kauf nehmen als nur über Schwierigkeiten grübeln...“.

Erfreulich war nicht nur der Erhalt der Veranstaltung, sondern auch der gewachsene Zuspruch an Teilnehmern und Beiträgen (s. Tab. 1). Das zunehmende Interesse an der Tagung und die Etablierung des Studienganges Ernährungswissenschaften an der Friedrich-Schiller-Universität Jena führten dazu, dass seit 1993 die Veranstaltung im zweijährigen Abstand in Jena stattfindet (s. Tab. 1).

Bei der Begrüßung zum 4. Symposium meinte Wolfram Braune, jetzt Prodekan der Biologisch-Pharmazeutischen Fakultät, zur Thematik Tagungsort und -frequenz: „...Auch in bezug auf den Tagungsort zeichnen sich Zielvorstellungen ab: Wenn Sie von Jena aus auf einer Landkarte die bisherigen Tagungsorte des Symposiums aufsuchen, erkennt man unschwer eine schrittweise, aber konsequente zentripetale Tendenz: Leipzig (Tagungsort der 1. Veranstaltung 1983) liegt etwa 100 km, Reinhardsbrunn (1987) etwa 70 km, Stadtroda (1991) 12 km vom heutigen Tagungsort Jena entfernt. Da die Träger des Symposiums jetzt diese Heimat gefunden haben, soll künftig auch Jena fester Tagungsort werden. (ich darf Sie aber beruhigen: es ist nicht vorgesehen, die Tagungsintervalle weiter exponentiell zu verringern)...“.

Trotz Frequenzerhöhung und eingeführtem Begutachtungssystem ist die Anzahl der Beiträge weiter angestiegen und hat sich zwischenzeitlich bei ≈ 100 stabilisiert. Um dem Untertitel „...bei Mensch und Tier“ gerecht zu werden, organisieren die Jenaer Kollegen seit 1995 das Symposium gemeinsam mit dem Institut für Tierernährung der Bundesforschungsanstalt für Landwirtschaft (FAL) in Braunschweig.

Tabelle 1: Übersicht zu den bisherigen Symposien „Vitamine und Zusatzstoffe“

Nr.	Jahr	Ort	Angemeldete Beiträge insgesamt	Beiträge zu Themen		
				Allgemein	Mensch	Tier
1	1983	Leipzig	31	9	11	11
2	1987	Reinhardsbrunn	60	9	3	48
3	1991	Stadtroda	84	5	6	73
4	1993	Jena	78	15	11	52
5	1995	Jena	103	16	30	57
6	1997	Jena	87	16	26	45
7	1999	Jena	108	18	45	45
8	2001	Jena	106	2	48	56
9	2003	Jena	105	12	40	53
10	2005	Jena	102	17	35	50
		Gesamt	864	119	255	490

Nach 1989 hatte sich die Problematik mit dem „nicht herauskommen“ oder wie Wolfram Braune im Jahre 1993 bei der Begrüßung erwähnte „...wenn der Berg nicht zum Propheten kam, muss der Prophet zum Berge kommen“, erübrigt.

An diese Stelle trat jedoch eine andere Aufgabe des Symposiums: Bedingt durch die Kontakte zu osteuropäischen Kollegen und in die Staaten der ehemaligen Sowjetunion wurde das Symposium auch zu einem Forum von Wissenschaftlern aus diesen Ländern, dankenswerterweise gefördert durch die Deutsche Forschungsgemeinschaft (DFG).

Da sich auf europäischer Ebene erhebliche Veränderungen bei Regulierungen zum Einsatz von Vitaminen und Zusatzstoffen ergeben haben, die überaus bedeutsam für die Wissenschaftler, die Hersteller von Zusatzstoffen und die Anwender sind, soll im Rahmen dieser Begrüßung nachfolgend kurz auf diese Entwicklungen unter besonderer Berücksichtigung der Futtermittelzusatzstoffe eingegangen werden.

Entwicklungen auf europäischer Ebene

Auf europäischer Ebene (Brüsseler Terminologie) werden unter dem Terminus Zusatzstoffe (Food and Feed Additives) essentielle (wie z. B. Aminosäuren, Spurenelemente, Vitamine) und nicht-essentielle (z.B. Enzyme, Mikroorganismen, organische Säuren, ätherische Öle) Substanzen zusammenfasst.

Nach der EG-Verordnung Nr. 1831/2003 werden unter Futtermittelzusatzstoffen Stoffe, Mikroorganismen oder Zubereitungen verstanden, die keine Futtermittel-Ausgangserzeugnisse oder Vormischungen sind und bewusst Futtermitteln oder

Wasser zugesetzt werden, um insbesondere eine oder mehrere der folgenden Funktionen zu erfüllen:

- Beschaffenheit des Futtermittels positiv beeinflussen
- Beschaffenheit der tierischen Erzeugnisse positiv beeinflussen
- Farbe von Zierfischen und –vögeln positiv beeinflussen
- Ernährungsbedarf der Tiere decken
- Ökologischen Folgen der Tierproduktion positiv beeinflussen
- Tierproduktion, Leistung oder Wohlbefinden der Tiere, vor allem durch Einwirkung auf Verdauungstrakt und Verdaulichkeit, positiv beeinflussen
- Kokzidiostatische oder histomonostatische Wirkung haben.

Nach dieser Verordnung werden die Zusatzstoffe in folgende fünf Kategorien eingeteilt:

- Technologische Zusatzstoffe (Konservierungsmittel, Antioxidationsmittel, Emulgatoren, Stabilisatoren, Verdickungsmittel, Geliermittel, Trennmittel und Säureregulatoren),
- Sensorische Zusatzstoffe (Farbstoffe, Aromastoffe und appetitanregende Stoffe).
- Ernährungsphysiologische Zusatzstoffe (Vitamine, Spurenelemente, Aminosäuren sowie deren Salze und Analoga, Harnstoff und andere NPN-Verbindungen),
- Zootechnische Zusatzstoffe (Substanzen, die die Nährstoffverdaulichkeit verbessern, Mikroorganismen oder andere definierte Substanzen, die die Mikrobiota im Verdauungstrakt in einer für das Tier günstigen Weise beeinflussen und Wachstumsförderer),
- Eine weitere gesonderte Kategorie sind Kokzidiostatika und Histomonostatika, welche krankheitsvorbeugende Mittel sind und zur Abtötung oder Wachstumshemmung von Protozoen eingesetzt werden.

Die fachliche Bewertung der Wirksamkeit und Sicherheit dieser Zusatzstoffe war bis 2003 bei der GD SANCO der EU und den acht wissenschaftlichen Ausschüssen (Scientific Committee's) dieser GD. Für Lebensmittelzusatzstoffe war beispielsweise das Scientific Committee on Food (SCF), für Futterzusatzstoffe das Scientific Committee on Animal Nutrition (SCAN) zuständig. Die Umsetzung der Empfehlung der wissenschaftlichen Ausschüsse in europäisches Recht erfolgte durch die EU-Kommission.

In Verbindung mit der BSE-Krise wurde im Jahre 2000 das Weißbuch zur Lebensmittelsicherheit der EU herausgegeben. In diesem Weißbuch wurden eine Trennung von Risikobewertung und Risikomanagement auf EU-Ebene und die Gründung einer Europäischen Behörde für Lebensmittelsicherheit (European Food Safety Authority, EFSA) vorgeschlagen. Die EFSA nahm 2002 ihre Tätigkeit zunächst in Brüssel auf und zog zum 01.10.2005 nach Parma, Italien, um.

In der EFSA beraten je 21 Experten aus allen europäischen Ländern in folgenden 8 Ausschüssen (Panels) zu verschiedenen Sicherheitsfragen:

- Food additives, flavourings, processing aids, material in contact with food (AFC)
- Additives and products in animal feed (FEEDAP)
- Plant health, plant protection products (PPR)
- Genetically modified organisms (GMO)
- Dietetic products, nutrition and allergies (NDA)

- Biological hazards (BIOHAZ)
- Contaminants in the food chain (CONTAM)
- Animal Health and Welfare (AHAW)

Für Fragen der Lebens- und Futtermittelsicherheit sind vor allem die AFC- und FEE-DAP-Panels zuständig. Dabei geht es nicht vorrangig um Effizienzbewertung der verschiedenen Substanzen, sondern um Sicherheitsbewertungen für Mensch (consumer, producer, user), Tier und Umwelt.

Die bisher von den Panels verabschiedeten Stellungnahmen sind auf der EFSA Website www.efsa.eu.int zugänglich. Diese Gutachten dienen der EU-Kommission für weitere Entscheidungen.

Neben der Sicherheitsbewertung verschiedener Substanzen anhand von eingereichten Dossiers ist die Erarbeitung von Richtlinien (Guidelines/Guidance Documents) für die Zusatzstoffherstellende Industrie besonders bedeutsam. In diesen Richtlinien wird vorgegeben, welche Studien für eine umfassende Sicherheitsbewertung der verschiedenen Zusatzstoffe erforderlich sind.

Überaus erfreulich ist auch, dass die Mitglieder der Panels Vorschläge für „Self Tasking Activities“ unterbreiten können. Dadurch werden nicht nur die offiziellen Anfragen der EU-Kommission beantwortet, sondern es können auch Themen bearbeitet werden, wenn aus wissenschaftlicher Sicht diese oder jene Problematik ansteht bzw. zu erwarten ist (z.B. Richtlinien zur Bewertung von Detoxifikationsmitteln von Mykotoxinen; Richtlinien zur ernährungsphysiologischen und Sicherheitsbewertung von Lebens- und Futtermitteln aus gentechnisch veränderten Pflanzen der zweiten Generation, Richtlinien zur Bewertung von Kräutern, ätherischen Ölen und anderen pflanzlichen Produkten als Zusatzstoffe).

Die Mitglieder der EFSA-Panels werden für drei Jahre aus weltweiten Bewerbungen berufen. Die nächste Periode beginnt im Mai 2006 und läuft bis April 2009.

Wünsche an die Forschung

Die Begrüßungsworte sollen auch genutzt werden, um einige Wünsche an die Forschung auf diesem spezifischen Fachgebiet zu formulieren.

Sicherlich könnte man hier aufschreiben, welche Erwartungen und Wünsche an die zukünftige Forschung mit Vitaminen und weiteren Zusatzstoffen zu stellen sind, wie z.B.

- Wesentliche Beiträge zum Erhalt und zur Stabilisierung der Gesundheit von Mensch und Tier
- Detoxifikation von unerwünschten Substanzen in Lebens- und Futtermitteln
- Beiträge zur effektiven Konvertierung der Futtermittel in Lebensmittel tierischer Herkunft bei minimalen Ausscheidungen in die Umwelt

In der jüngsten Vergangenheit häufen sich jedoch auch die Wünsche nach umfassender Publikation aller mit den verschiedenen Zusatzstoffen in Experimenten erzielten Befunde, auch wenn keine „positiven“ Effekte oder gar „negative“ Ergebnisse erzielt wurden. Das Öffentlichmachen aller Versuchsergebnisse ist die Vorausset-

zung für eine vergleichende Bewertung und die Einschätzung der Wirkungsweise der jeweiligen Substanz. „Publikationspflicht“ wäre auch die Basis für Übersichtsarbeiten einschließlich des Literaturteiles von Dissertationen auf diesem Gebiet. Dieser Appell richtet sich sowohl an die Wissenschaftler als auch an die Herausgeber wissenschaftlicher Zeitschriften.

Gegenwärtig ist einzuschätzen, dass die Bedingungen, bei denen verschiedene nicht-essentielle Zusatzstoffe oder „Vitamin-Hochdosierungen“ mit Sicherheit bestimmte erwartete Wirkungen haben, nicht immer eindeutig beschrieben werden bzw. vorhergesagt werden können.

Notwendig ist demnach mehr Forschung bezüglich der Aufklärung der Wirkmechanismen verschiedener Zusatzstoffe. Dadurch wird es möglich, die Zusatzstoffe gezielter einzusetzen und die Ergebnisse mit größerer Sicherheit zu reproduzieren. Es ist kaum zu erwarten, dass derartige Forschungen zukünftig mit öffentlichen Mitteln erfolgen können. Neue Wege der Forschungsfinanzierung, z. B. über eine Umlagefinanzierung interessierter Partner (z. B. zusatzstoffherstellende Industrie, Mischfutterindustrie, Milchindustrie, Bauernverband) erscheinen dringend erforderlich.

In Diskussionen mit amerikanischen Kollegen über Sinn oder Unsinn des Einsatzes verschiedener Zusatzstoffe erhält man oft die Antwort, dass „der Einsatz derartiger Substanzen dann sinnvoll ist, wenn mit großer Wahrscheinlichkeit der Nutzen größer ist als der Schaden“. Damit solche Statements durch wissenschaftliche Befunde ersetzt werden können, erscheint mehr Forschung auf dem Fachgebiet notwendig.

Abschlussbemerkung

Wie bereits bei den letzten Symposien haben wir eine Dreiteilung der Beiträge vorgenommen:

- Plenarbeiträge
- Kurzvorträge
- Poster

Wir hoffen, dass die nun als Tagungsband vorliegenden Beiträge Ihnen Impulse für die eigene Arbeit vermitteln.

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Interactions between vitamins and trace elements

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Abstract

Bioavailable iron and vitamin A are mainly provided in the human diet by animal source foods. In the poorer populations of the developing world, consuming mainly plant-based diets, deficiencies of these micronutrients are common and can coexist in the same individual. In addition, in areas where soil iodine is low, iodine deficiencies can also occur. Infants, children as well as pregnant and lactating women are most at-risk of deficiency because of their extra requirements for growth. About 250 million pre-school children are said to be vitamin A deficient based on low serum retinol values. Women of child-bearing age are especially at risk of iron deficiency because of monthly blood losses during menstruation. On the basis of low ferritin concentrations, it is estimated that about 2 billion people worldwide are iron deficient and that one billion of these iron deficient people are thought to suffer from iron deficiency anemia, the most severe form of iron deficiency. Based on low urinary iodine levels 1.9 billion people are estimated to have inadequate iodine nutrition and 200 million suffer from goitre.

The influence of vitamin A deficiency on both, iron and iodine metabolism is discussed. Low vitamin A status has long been known to promote anemia although the mechanism is still unclear. Recent iron absorption studies have further complicated the issue as vitamin A added to iron-fortified foods has been shown to increase, decrease or have no effect on iron absorption depending on the subject. Recently, it has been also suggested that vitamin A supplements may have a negative impact on iodine metabolism in iodine deficient subjects. Finally the influence of iron deficiency on iodine metabolism is discussed and food fortification studies demonstrating the beneficial effect of iron on iodine utilization are described.

Micronutrient deficiencies – the significance of interactions between vitamins and trace elements

The three major micronutrient deficiencies worldwide are iron, iodine and vitamin A and they remain a major public health problem. The latest WHO figures suggest that about 2 billion people are iron deficient and about 1 billion are affected by iron deficiency anemia (IDA) [1]. Despite high efforts with iodized salt worldwide, 633 million people are still estimated to be goitrous [2], and 250 million pre-school children are estimated to be vitamin A deficient (VAD) [3].

Iron deficiency

Consequences of iron deficiency depend on the severity of the condition. The spectrum includes fatigue, weakness, increased susceptibility to infections, diminished work capacity, increased maternal and perinatal mortality, increased prevalence of preterm and low-birth weight infants, and reduced cognitive development and reduced learning ability of children. Two billion people are iron deficient as estimated by WHO, making every third person inadequately supplied with dietary iron.

Vitamin A deficiency

VAD is a major public health problem in many developing countries, particularly those in Africa and South-East Asia. VAD is the leading cause of preventable blindness. In children, VAD also significantly increases the risk of severe illness and death from childhood infections such as diarrheal disease and measles [4, 5]. Each year up to 500 thousand children become blind and 50 % of these children die within 12 months of losing their sight.

Iodine deficiency

Inadequate intake of iodine impairs thyroid function and results in a spectrum of disorders - goiter, cognitive impairment, and congenital abnormalities - collectively referred to as the iodine deficiency disorders (IDD). IDD affects over 740 million people or 13% of the world's population; 30% of the remainder are at risk of iodine deficiency. Iodine deficiency is the world's most prevalent – yet easily preventable – cause of brain damage [6, 7].

The two major risk factors for micronutrient deficiencies are low intake or absorption of the target micronutrient and increased requirements, especially of the vulnerable popu-

lation groups such as infants, children and young women in lower socioeconomic populations of Africa, South Asia and Latin America. In these regions of the world, the diet of the lower socioeconomic populations is of low quality. In addition, micronutrient requirements are greatly increased during growth of children, during pregnancy and with infection. Menstruating women also have a much greater demand for iron than other population groups.

In the past, it has been the practice to evaluate the epidemiology of micronutrient deficiencies separately for each micronutrient and to develop individual strategies for their prevention or treatment. However several micronutrient deficiencies can occur simultaneously in the same individual from those population groups eating low quality diets based on plant foods with little meat. In these populations, single micronutrient deficiencies rarely occur and multiple deficiencies should be expected and taken into account when developing prevention strategies. When there are overlapping micronutrient deficiencies in the same individual, there is a potential for interactions. This review looks at 3 such potential interactions, iron and vitamin A, vitamin A and iodine and iron and iodine [8-11].

Iron and vitamin A

The low intake of vitamin A from animal source foods or a low intake and/or bioavailability of provitamin A carotenoids from plant foods can result in a low vitamin A status. Vitamin A deficiency and anemia have been linked for many years but the mechanism by which vitamin A exerts its effect remains unclear. The most common explanation for the interaction is an influence of vitamin A on iron metabolism [12]. In addition, vitamin A deficiency can decrease immune function, increase infections and the associated anemia which is due to a modulation of hematopoiesis [13]. In such a situation, an increased intake of vitamin A or bioavailable pro vitamin A carotenoids resulted in an increased iron utilization and an improved iron status.

Early human studies [14-16] reported anemia together with hemosiderosis of the spleen and liver in vitamin A deficient subjects. On vitamin A repletion there was a regeneration of bone marrow, disappearance of haemosiderin from the spleen and the liver, and an increased erythroblastic activity. Hodges *et al.* [17] later showed that providing high iron

supplements to subjects with both iron and vitamin A deficiency did not increase Hb levels. In this study, 8 middle-aged men were fed a combination of 3 different vitamin A deficient diets for 360-770 days. Despite a high daily intake of iron (18-19 mg), the men developed mild anemia after about 6 months and the anemia was not responsive to iron therapy until the subjects were repleted with vitamin A.

Many cross-sectional studies in developing countries have reported a positive correlation between serum retinol and hemoglobin concentration, in spite of the influence of other nutritional factors and infectious diseases. Intervention studies have additionally shown that vitamin A supplements, or foods fortified with vitamin A, improve blood hemoglobin concentrations in children and in pregnant or lactating women [18]. In addition, some studies have shown that dual supplementation of iron with vitamin A has a greater impact on hemoglobin concentrations than iron alone, in both children [19] and pregnant women [20]. However, some studies have shown no significant effect of vitamin A on hemoglobin concentrations, which might be expected especially if the vitamin A status is adequate [21].

Possible mechanisms

Iron metabolism can largely be described within the red cell cycle which represents primarily the formation and destruction of red blood cells. Small amounts of iron enter the cycle via absorption of dietary iron and an equivalent amount of iron is lost from the blood and tissue. Several mechanisms have been suggested for the influence of vitamin A deficiency on iron metabolism (Figure 1).

The influence of vitamin A on erythropoiesis might be due to a reduced iron incorporation into red blood cells via a decreased mobilization of ferritin iron from the spleen or liver [22], to a decrease in iron absorption [23] or due to an ineffective erythropoiesis [12]. Alternatively it could be indirectly due to a improved immune function and a decreased anemia of infection [13]. Infection is reported to block iron absorption [24]. So it is possible that the immune promoting properties of vitamin A may improve iron absorption by reducing inflammation. Also there is reported to be a vitamin A responsive element on the erythropoietin gene [25].

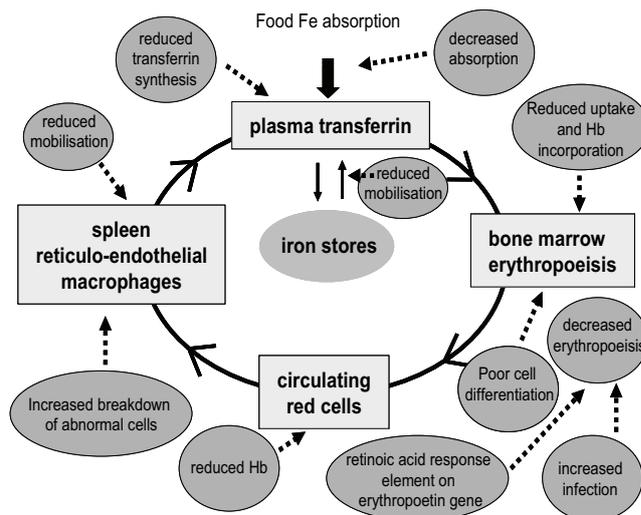


Figure 1: Possible influences of vitamin A on iron metabolism

These theories are largely based on rat studies where vitamin A deficiency has been reported to reduce the incorporation of radioactive iron into the erythrocytes by almost 50%, alter red cell morphology, produce mild anemia, lower plasma total iron binding capacity and per cent transferrin saturation but not circulating transferrin concentrations, and cause an accumulation of iron in the liver, spleen and the bone marrow. An increased iron absorption from the gut was reported in Vitamin A deficient rats, which would not really support the observation that vitamin A increase iron absorption in humans [26-28].

Based on the results from these animal studies, Roddenburg *et al.* hypothesized that vitamin A deficiency impairs erythropoiesis so that mild anemia with malformed cells develops [12]. The abnormal erythrocytes would be broken down by the macrophages of the reticulo-endothelial system at an increased rate and so help explain the accumulation of iron in the spleen. Roodenburg *et al.* [12] however could find no evidence in rats

that vitamin A deficiency effects erythropoeisis per se and speculated that iron accumulation in the spleen may be related to a reduced iron transport due to an inhibition of transferrin synthesis, although Mejia & Arroyave [29] found no decrease in circulating transferrin concentrations in vitamin A deficient rats. Nevertheless, it is possible that vitamin A is involved in the release of iron from the spleen or the liver stores, or perhaps directly in the incorporation of iron into hemoglobin.

Human studies investigating the influence of vitamin A on iron absorption have generated contradictory results. In Venezuela, it has been reported in a series of studies with radioiron isotopes that vitamin A or β -carotene increase iron absorption by poor Venezuelan subjects from iron fortified wheat bread, maize bread and rice meals (Table 1) [23, 30].

Table 1: Influence of Vitamin A on iron absorption from a maize bread breakfast [23]

	% iron absorption ^c \pm SEM	
Study 1		
maize bread	5.8 \pm 1.1	p < 0.05
maize bread + coffee	2.0 \pm 1.2	
maize bread + vitamin A + coffee	8.5 \pm 1.2	p < 0.05
Study 2		
maize bread	3.2 \pm 1.4	p < 0.05
maize bread + vitamin A	6.3 \pm 1.3	
maize bread + tea	2.0 \pm 1.2	p < 0.05
maize bread + vitamin A + tea	3.6 \pm 1.1	

^a Maize bread contained 5mg iron/100g as ferrous fumarate and, when vitamin A fortified, contained 330 μ g RE/100g (about 80% losses)

^b Bread was fed with margarine and cheese

^c Includes both iron absorption and iron incorporation into hemoglobin

However, using a similar radioiron methodology, as well as a stable isotope technique, Walczyk *et al.* [31] found no influence of vitamin A on iron absorption from iron fortified maize bread fed to more affluent Swiss and Swedish students. To further complicate the issue, it was recently reported that vitamin A added to a maize gruel fed to children in the Côte d'Ivoire, who were both vitamin A and iron deficient, inhibited iron absorption [32]. In the same children, there was no effect of vitamin A on iron absorption 3 weeks after they had been supplemented with high dose of vitamin A. Although Layrisse *et al.* [23] proposed that vitamin A and beta-carotene somehow act as iron chelators and prevent iron combining with inhibitors of iron absorption, such as phytate and polyphenols; this seems unlikely due to the low amounts of vitamin A in relation to iron. The difference in bioavailability in the above studies would appear to be subject related.

Iodine and Vitamin A

Vitamin A deficiency (VAD) and iodine deficiency affect >1/3rd of the global population [6, 7]. These deficiencies often coexist in children. In rural Côte d'Ivoire, 32-50% of school-age children suffer from both VAD and goiter [33]. In northern Morocco, 41% of children have VAD and 50% are goitrous [34].

Several cross-sectional studies have investigated the relationship between VAD and thyroid function or goiter. In vitamin A-sufficient Senegalese adults, there was a strong negative correlation between increasing severity of goiter and serum retinol and retinol transport proteins (retinol-binding-protein RBP and transthyretin TTR) [35, 36]. In mildly vitamin A-deficient Ethiopian children, those with visible goiters (grade IB or II) had significantly lower serum retinol and RBP than children without or with grade IA goiter [37]. In these children, serum thyroid stimulating hormone (TSH) was normal, and there was a negative association between thyroid binding globulin (TBG) and the triad of retinol, RBP, and TTR.

In Ethiopian children with clinical signs of severe VAD, serum TSH was normal, and TT3 (but not TT4) was significantly correlated with retinol, TTR, and albumin [38]. However, in these studies it was not possible to distinguish the effects of VAD from protein malnutrition, which can also reduce serum retinol and RBP [39]. Among adequately nourished 7–14 year-old children in the 1987 National Nutrition Survey in the Philippines, the

prevalence of goiter was 1.8% in children without VAD and 5.3% in those with VAD [40]. These studies clearly demonstrate a link between vitamin A status and thyroid function. VAD is linked to higher goiter rate, and ID is linked to lower vitamin A status (lower serum retinol and RBP).

Possible mechanisms

Early animal studies have shown inconsistent in relation to the impact of vitamin A deficiency on thyroid metabolism; they have been reviewed by Hess and Zimmermann in 2004 [8]. Recent findings suggest that VAD may affect thyroid metabolism through a central mechanism (Figure 2). Vitamin A deficiency may play a role in the pituitary and the regulation of TSH β -subunit production.

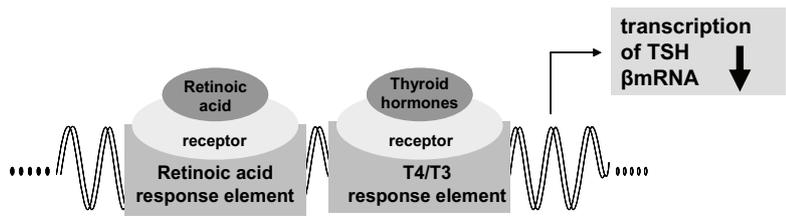


Figure 2: Ample supply of thyroid hormones and vitamin A suppress transcription of TSH β mRNA (modified from Wolf *et al.* [41])

TSH is the key hormone in stimulating the pituitary producing thyroid hormones. It is built of two subunits: the α -subunit is unspecific and is part of several hormones; the specific subunit is the β -subunit. The ample supply of thyroid hormones and retinoic acid suppresses TSH β -subunit production. Both the thyroid hormone-activated thyroid receptor and the 9-cis-retinoic acid-activated retinoid X receptor suppress transcription of the pituitary TSH β gene by occupying half-sites on the promoter DNA of the gene [41, 42].

A depletion of liver retinol stores may lead to a decreased activity and expression of liver UDP-glucuronosyltransferase (UGT). The role of UDP-glucuronosyltransferase in detoxification and elimination of thyroid hormones and other endogenous substrates has been investigated by several authors [43, 44].

The effect of VAD on thyroid metabolism may be mediated at least partly through shared transport proteins. Transthyretin (TTR) binds 10-15% of circulating thyroid hormone [45]. TTR is also the primary indirect carrier of vitamin A (all-trans-retinol) in the plasma through its interaction with retinol-binding protein (RBP), the vitamin A transport protein [46]. Although VAD decreases hepatic release of RBP, release of TTR is similar during vitamin A depletion and repletion in rats. Serum TTR concentration is therefore unchanged by VAD [47, 48].

Recent studies

Recent findings in Morocco showed that moderate Vitamin A Deficiency (VAD) in severely iodine-deficient subjects may reduce risk for hypothyroidism [49]. The findings indicate that VAD in severely-IDD-affected children increases TSH stimulation and thyroid size, and reduces risk for hypothyroidism. This effect could be due to decreased VA-mediated suppression of the pituitary TSH β gene. In IDD and VAD-affected children receiving iodized salt, concurrent vitamin A supplementation improved iodine efficacy. The data are consistent with the possibility that VAD may decrease activation of the pituitary retinoid receptor, thereby increasing transcription of the TSH β gene and increasing TSH secretion. Increased TSH stimulation of the thyroid increases thyroid size but maintains circulating thyroid hormone, protecting against hypothyroidism. Results from a recent study looking at the impact of concurrent vitamin A and iodine deficiencies on the thyroid-pituitary axis in rats in our group showed that combined deficiencies of vitamin A and iodine have a greater impact on thyroid status as compared to the single deficiencies. The significant decrease in thyroid weight and serum TSH after supply with vitamin A without concurrent iodine repletion in depleted rats suggests that there may be a risk of thyroid insufficiency associated with supplementation of vitamin A in combined deficiencies (Biebinger *et al.* unpublished).

Iron and iodine

In regions of West and North Africa, 23 to 35% of school-aged children suffer from both goiter and IDA [50, 51]. Iron deficiency with or without anemia can have adverse effects on thyroid metabolism. IDA may have a greater impact on IDD than previously described goitrogens because of its high prevalence in vulnerable groups [52].

Data from the few available cross-sectional studies that have investigated the correlation between IDD and IDA are equivocal. A survey in Ethiopian children found no correlation in goiter rate or thyroid hormone levels and iron status [37]. Also, no significant difference was found in the prevalence of anemia between goitrous and nongoitrous subjects in the Philippines [40]. However, in severely vitamin A-deficient Ethiopian children, low levels of T3 were associated with serum iron and low transferrin saturation [38]. A national screening in 2917 children in Iran also reported a highly significant difference in goiter rates by palpation between children with low and normal SF levels [53]. Goiter was 3.8 times more prevalent in school children with low SF levels than in children with normal SF concentrations.

Moreover, Zimmermann *et al.* [50] assessed iron status and goiter rate by palpation in 419 children aged 6–15 years in two villages in western Côte d'Ivoire and found a relative risk of 1.9 (confidence interval 1.5–2.3) for goiter for children with IDA. To investigate the impact of iron supplementation in goitrous, iron-deficient children Zimmermann *et al.* designed a trial of oral iodized oil followed by oral iron supplementation in an area of endemic goiter in the western Côte d'Ivoire [54]. One hundred and four goitrous Ivorian children, divided in 2 groups (non-anemic and IDA) received a single oral dose of 200 mg iodine (Lipiodol®) and were followed for 30 weeks. The prevalence of goiter after 30 weeks was 12% in group I and 64% in group II. A strong correlation was found between the percentage decrease in thyroid volume and hemoglobin concentration ($r^2 = 0.65$) in anemic children. After receiving iron supplements from 30 weeks to 42 weeks, the goiter prevalence in the anemic children fell to 31% and 20% at weeks 50 and 65 (Figure 3).

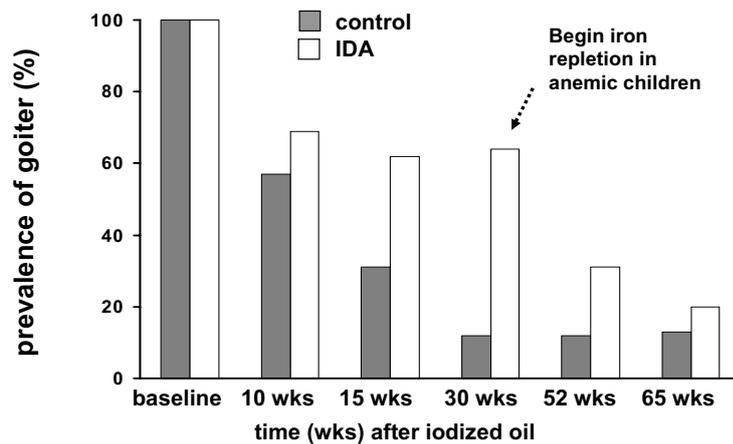


Figure 3: Influence of IDA on iodine utilization and goiter prevalence at baseline and after iron treatment of IDA children from 30-42 weeks [50, 54]

Potential mechanisms of the iodine and iron interaction

Certain aspects of thyroid metabolism in iron deficiency overlap with those observed in hypothyroid states. The plasma concentrations of T4 and T3 are lower and thyroid response to several different input stimuli is blunted in IDA. However, it is not clear how iron deficiency exerts its effects on thyroid and iodine metabolism. Beard *et al.* [55] suggested that IDA induces alterations in central nervous system control. Normalization of plasma T4 kinetic parameters in iron-deficient anemic rats provided with exogenous T4, suggests that low plasma T4 concentrations contribute to the altered thyroid hormone kinetics associated with iron deficiency [55]. Presumably, in iron-deficient anemic rats, a smaller portion of T4 is converted to T3 and a larger portion is converted to reverse T3, a physiologically inactive metabolite. This is in agreement with a study by Smith *et al.* [56] who concluded that iron-deficient rats are functionally hypothyroid, with a tendency toward thyroid hormone inactivation versus activation. According to Beard *et al.* [10] the effect of iron deficiency on either the hepatic 5'-deiodinase or the brown fat deiodinase II observed in rats is rather minimal.

Thyroid impairment was also found in chronically hypoxic children, who had not only increased levels of reverse T3, but also decreased concentrations of T4 and T3, whereas in acutely hypoxic children, mean serum T4 and T3 concentrations were not altered, but mean serum reverse T3 concentration was significantly elevated [57]. However, in healthy subjects, hypoxic stress led to marked elevations in plasma T4 and T3 within 4 hours and the increased levels were maintained during the entire period of exposure [58]. This indicates that in the healthy subject, hypoxia cannot entirely explain hypothyroidism associated with IDA. The association between anemia and hypothyroidism may be physiologic to some extent; that is, a result of reduced need for delivery of oxygen to peripheral tissues in hypothyroidism [59]. On the other hand, a widely recognized effect of thyroid hormones is their influence over energy metabolism [60]. As food intake is reduced in anemia, falling thyroid hormone concentration may be in part a physiologic adaptation. This has been confirmed by reduced thyroid hormone concentrations in modified fasting of rats [61].

An additional mechanism that could induce increased thyroid volume in IDA is the interaction of nitric oxide with Hb. Nitric oxide is a potent vasodilator that is produced in endothelial cells and has been assumed to act exclusively at its site of synthesis [62, 63].

Finally, TPO is a glycosylated heme-enzyme bound to the apical membrane of the thyrocytes [64]. It plays a key role in thyroid hormone synthesis as it catalyzes the two initial steps, iodination of the thyroglobulin (Tg) and coupling of the iodotyrosine residues [65]. While the thyroid hormone synthesis occurs at the apical membrane of the thyrocytes, TPO is localized in the endoplasmic reticulum and in the perinuclear membrane. We have recently shown in rats that TPO activity is significantly reduced in IDA (Figure 4) [66].

Male weanling Sprague-Dawley rats ($n = 84$) were assigned to seven groups. Three groups (ID-3, ID-7, ID-11) were pair-fed iron-deficient diets containing 3, 7, and 11 μg iron/g. An iron-sufficient diet was consumed *ad libitum* by one control group. After four weeks, Hb, T3, and T4 were significantly lower in the iron-deficient groups than in the control group ($p < 0.001$). TPO activity (by both guaiacol and iodide assays) was markedly reduced by iron deficiency ($p < 0.05$). Compared to the *ad libitum* control group,

TPO activity per total thyroid determined by the guaiacol assay in the ID-3, ID-7, and ID-11 groups was decreased by 56, 45, and 33%, respectively ($p < 0.05$).

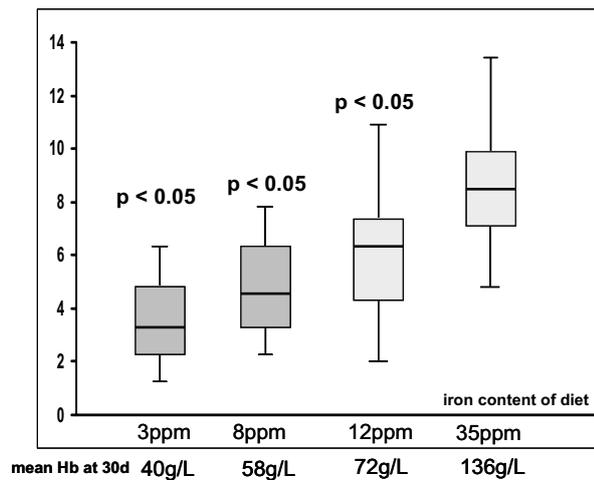


Figure 4: TPO activity in thyroid of iron deficient rats (n=12), guaiacol units per thyroid [66]

Conclusions

Iron, iodine and vitamin A deficiencies are common in the developing world, especially in infants, children, adolescents and pregnant women, all of whom have proportionally higher requirements due to growth. Multiple deficiencies may occur in the same individual. Interactions have been reported between iron and vitamin A, iodine and vitamin A, and iron and iodine and these interactions must be considered when planning intervention strategies such as supplementation or fortification; since single nutrient interventions may be less effective or, in the case of vitamin A supplementation to iodine deficient children, have a potential negative impact.

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The contribution of fortified foods to micronutrient intake in Austria

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Despite the availability of adequate amounts of food, malnutrition is still a problem in western industrialised countries. One of the major nutrition related problems in Europe is overweight and obesity, but this does not mean that undernourishment with certain nutrients does not occur.

The micronutrients intake in European countries appear to be adequate for most nutrients. However, a number of population subgroups are at higher risk of suboptimal intakes for folate, vitamin D, iodine, iron, and calcium. Dietary surveys indicate that food fortification can play a role in mitigating such risks for some nutrients [ENHR, 2004].

In Austria, population groups at risk are especially young women at childbearing age, pregnant women and elderly people [Elmadfa et al., 2003].

Fortification means the addition of nutrients to food and beverages. The basis of the enrichment may be the restoration of nutrient losses due to food processing and storage or the addition of one or more nutrients whether or not it is normally contained in the food [Codex Alimentarius, 1987]. The aim of food fortification is to prevent or correct nutrient deficiency in the whole population or in subpopulation groups at risk [WHO/FAO, 1994]. In recent years the enrichment of food has become another "functional" aspect. The research no longer just focuses on preventing nutrient deficiencies and non-communicable diseases but tries to improve the health benefit of our food.

There is a great range of foods serving as fortified products; staple food as well as products for special population groups, beverages and sweets. There is also a variety of fortifying agents, which are vitamins, minerals and other micronutrients.

Staple food is most commonly used for mandatory regulated fortification and adding nutrients to food is since decades common practice. In Austria e.g. the iodine fortification of salt began in 1924 and became mandatory in 1963. The contribution of fortified salt to iodine intake of children is about 50% in Austria [Elmadfa et al., 2003]. Other countries like UK and USA add nutrients to grain products. In some northern

countries the enrichment of milk and margarine with Vitamin A and D has been implemented.

There are some studies showing positive effects of mandatory fortification. In the US folate fortification has resulted in an almost 20% reduction of neural tube defects [McNulty, 2001]. Also in USA and Ireland adding iron to flour reduced iron deficiency and iron deficiency anaemia in children and pregnant women.

There is a broad range of fortified food on the market. In the European Union the market share of fortified food is estimated of about 1-6%. A survey conducted in five EU member states showed that 50% of the household shoppers buy fortified products. 78% believe that consumers should have the right to choose foods with nutrients added [Capibus Europe, 1999].

The introduction of products enriched with nutrients with a relatively low tolerable upper level of intake (e.g. iron) has raised safety concerns about a potential risk for high consumers. Therefore analysis of the market range and the average intake of fortified foods were required.

An inspection of supermarkets and retail outlets in Austria showed 468 different fortified products. They could be grouped in baby food, beverages, sweets, beverage powder, cereals, dairy products, edible fats, salt and miscellaneous. Baby food contributes the most, followed by beverages and sweets. The study also analysed how many nutrients have been added to a product. Altogether 29 different micronutrients were usually used for fortification. About a third of the products were enriched with 6 to 10 of these nutrients. Around 10% even contained more than 10 different micronutrients.

A big part of fortified foods is not designed for special target groups but for an average consumer. About 30% address parents (baby food) and 18% are considered for children [Wagner et al., 2005].

Asking consumers in Austria whether they buy fortified foods or not, more than 62% admit. The frequency of most fortified products consumed is according to food frequency questionnaires low or hardly ever [Elmadfa et al., 2003].

Fortification regulations differ from country to country, also within the European Union. In the United States fortification is regulated by the FDA whereas within the EU countries fortification regulations are liberal. The Codex claims that nutrients should not be added unless a need is documented [Meltzer et al., 2003]. In Austria therefore

fortification is liberally allowed as long as there are no adverse health effects to be expected.

The extent in which vitamins are added to food ranges from 25 up to 100% of the recommended dietary allowances (RDA). The amounts of micronutrients added to e.g. milk products or cereals show a great variance. The most frequently added nutrients are vitamin C, B vitamins, vitamin E and calcium. Trace elements and macronutrients are seldom added.

There is no doubt that fortified foods contribute a good deal to the daily micronutrient intake. The missing regulation leads to nonselective fortification, meaning that most of the added nutrients don't align with population needs. Based on the reference values for the German speaking countries Germany, Austria and Switzerland (D-A-CH reference values) the contribution of fortified products for the daily requirements has been assessed. In Austria 40% of the recommended vitamin C intake comes from enriched products. Regarding some vitamins of the B-group, there is a range of intake from 20% for vitamin B12 up to 37% for vitamin B6 deriving from enriched food. Folate intake is covered only 10% by fortified products.

Minerals are less often added and so the intake from fortified foods is also minor. Iron deriving up to 10% from enrichment supplies most, but still not enough for specific groups at risk. Considering only regular users of fortified products the picture changes seriously. The nutrient ranking stays the same but the contribution is much higher. Three quarters of the vitamin C and between 37% and 71% of the B-vitamins are ingested through fortified foods. Around 20% of folate derives from added nutrients mostly from the consumption of cereals and bran.

The data indicates no risk of overdose. Only for high consumers (95th percentiles) the upper safe level of intake was reached for vitamin A [Wagner et al., 2005].

Table 1: Average daily intake of micronutrients from fortified foods in % of the D-A-CH reference values, age group 25-51 years, n=1,700; (source: Austrian Nutrition Report 2003)

nutrient	in % of reference	nutrient	in % of reference	nutrient	in % of reference
Vitamin C	40	Vitamin B ₁₂	20	Copper	8
Vitamin B ₆	37	Vitamin E	17	Calcium	8
Niacin	29	Vitamin A	11	Magnesium	7
Vitamin B ₁	27	Folic acid	10	Zinc	4
Vitamin B ₂	23	Vitamin D	2	Iodine (excl. salt)	2*
Pantothenic acid	22	Iron	10	Fluoride	1

Fortified foods target not only at risk groups, but also at healthy individuals. They are widely implemented in our daily eating patterns. Although they may contribute to a well-balanced diet they cannot replace whole grain products, vegetables and fruits to ensure good health. Food fortification is one possibility to optimize nutrition status if adjusted to the specific needs of the target group. The main target should still be to prevent or correct a deficiency or suboptimal intake within a population group. At the moment food fortification is arbitrary, regardless of the real needs, which makes it necessary to carry out risk analysis assessing too low or excess intakes of single micronutrients. Regarding the actual intake and fortification practice there is no evidence of an oversupply with single micronutrients in Austria.

Abstract

In general, micronutrient intake in European countries appears to be adequate for most nutrients. However, a number of population subgroups are at higher risk of suboptimal intakes for folate, vitamin D, iodine, iron, and calcium. Dietary surveys indicate that food fortification can play a role in mitigating such risks for some nutrients.

Nowadays the interest in fortified food is constantly increasing among consumers as well as food producers. According to this, the range of fortified products in the market is expanding. Safety concerns are addressed in relation to the potentially increased level of nutrients with relatively low tolerable upper intake levels (e.g. iron).

The Institute of Nutritional Sciences thus carried out studies about fortified foods. An evaluation in supermarkets showed that 73% of nearly 500 different fortified products existing on the Austrian market were fortified with vitamin C. Vitamin C was thus the most frequently used nutrient although the vitamin C status can be regarded as good. Other vitamins and minerals were used less often. Most fortified foods were not adapted to the needs of specific target groups.

Food fortification is an effective tool for increasing the intake of some vitamins. The study population met e.g. 40% of the vitamin C requirements from fortified products. Nevertheless, fortification of foods mostly occurs arbitrarily and special needs of vulnerable groups, such as elderly people, are rarely considered. The fortification with micronutrients, of which an increase of intake would be desirable (e.g. folic acid), is not sufficient. Other nutrients (e.g. niacin) are added in such a high amount which exceeds by far the required level of intake. However, the risk of an overdose of added nutrients is not given with the consumption habits of currently provided fortified foods.

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Fatty acids in animal nutrition: fragile equilibrium between benefit and harm

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Introduction

In the last decades, there was a permanent shift in perception of and in recommendations for and against individual fatty acids with respect to human health. Currently, low contents and proportions of medium-chain fatty acids and of most *trans* fatty acids, and high proportions of omega-3 (ω -3) fatty acids (respectively a low ω -6 : ω -3 ratio) and of conjugated linoleic acids (CLA) are recommended for human nutrition (reviewed by Kraft and Jahreis, 2004; Moser, 2004). There could be also health advantages of butyric acid (Chilliard et al., 2000) and phytanic acid (McCarty, 2000). However, approaches to improve the sensory appearance and processing quality of the fats are still economically more feasible than improvements of the dietetic value, since inappropriate qualities of meat, milk and eggs are rejected or prices are reduced in cases where payment schemes exist. This is particularly true with respect to firmness of pig carcass fat (Bee, 2004) and of butter in summer. Very often these goals are contradictory to those of products considered to be especially healthy. Mainly looking at only one of these goals, various attempts have been made in animal nutrition research to modify fat in the products according to demand. The aim of this review is to describe successful options for the enrichment of animal-derived products with desired fatty acids and their importance for the two contrasting goals outlined. Finally, potential options to deal with this problem are discussed as a basis of a more holistic view in determining the ideal fat or fatty acid supplementation of livestock.

Characteristics of animal fat with high dietetic value and that with desired sensory and processing properties

Health aspects of individual fatty acids

Fatty acids are an extremely sensitive issue with respect to the perception of the public of healthy food. Individual fatty acids experience ups and downs with the most recent judgement being the following: desired fatty acids are ω -3 fatty acids, in particular eicosa-pentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) and α -linoleic acid (ALA) (Moser, 2004), oleic acid, butyric acid (Chilliard et al., 2000; prevalent only in milk fat and only hardly to be modified directly

in its proportion) and, less clear (Kraft and Jahreis, 2004; Scheeder, 2004), CLA with *cis-9,trans-11-C18:2* being the isomer believed to be particularly beneficial for human health (Kramer et al., 1997) and phytanic acid (McCarty, 2001). Fatty acids dietetically not desired include the medium-chain fatty acids (MCFA; in particular lauric, myristic and palmitic acid), ω -6 polyunsaturated fatty acids (PUFA) and *trans* mono-unsaturated fatty acids (MUFA). Also in the *trans* MUFA a differentiation in the extent of an adverse effect, if any, is indicated, since for instance *trans*-vaccenic acid, the major C18:1-*trans* fatty acid from rumen fermentation, is sometimes even considered to be beneficial in terms of human health especially as it is one precursor of *cis-9,trans-11* CLA (Griinari et al., 2000). Mostly, effects of fatty acids (positive and negative) seem to be related to coronary heart diseases and cancer, except phytanic acid which is associated with anti-diabetic effects (McCarty, 2001).

Sensory and processing properties

The desired characteristics differ among and even within commodities. Spreadability of butter, for instance is a problem in winter, while low melting temperatures are a problem of butter in summer. Concerning pork and pork products, consumer's complaints with mostly include too soft fat, while beef and mutton may exhibit fat congealing on the plate before being consumed. Strategies aiming at firmer fat have to exclude unsaturated fatty acids as much as possible (Kreuzer et al., 1997), which is contradictory to the intention to enhance the proportion of most of the dietetically desired fats mentioned above.

Successful strategies to modify fats in animal-derived products

Basically, there are two different sources of animal feeds to manipulate the fatty acid composition of the fat in animal-derived products. The first is the preferential application of feeds in animal nutrition which result in an improved fatty acids profile of the products, sometimes also associated with certain types of production systems. Accordingly, grazing of dairy and beef cattle, for instance, is a strategy which nicely fits with animal welfare claims and is often associated with elevated contents of beneficial fatty acids. The second approach is strategically using desired (functional) fatty acids, precursors of them, or fats particularly rich in these fatty acids (e.g. fish oil, linseed) as feed additives. Most of the successful strategies described below are, however, aiming to fulfill only one goal, i.e., either increasing the dietetic value or the sensory and processing properties of the fats.

Feeding systems to improve fats in animal-derived products

Fat (iodine) number as applied in Swiss abattoirs

About two decades ago, the Swiss pork processing industry has introduced a quality payment system based not only on lean meat proportion but also on fat firmness as this was recognized as a prerequisite to produce e.g. salamis of a quality satisfactory to the consumers (Scheeder et al., 1999). Being presently carried out on randomly selected pig carcasses, there will soon be new methods available for on-line measurement at the slaughter line of the so-called fat number (which is basically a iodine number; Scheeder et al., 1999) by infrared technique (Schwörer, 2004). One side-effect of this payment scheme was a deceleration of Swiss pig breeding towards excessively lean carcasses, since decreasing fat tissue increases the occurrence of PUFA (De Smet et al., 2004) as the ones indispensable for tissue cell function. As the PUFA and the MUFA are particularly effective in impairing fat firmness in pigs (Gläser et al., 2004), another response of the pig producers and the feed industry was strategic feeding (including a novel, but so far not fully accepted, concept called MUFA:PUFA norm; Bee, 2004). This banned almost all oils and oil-containing feeds and even provoked pressure on barley breeders to identify cultivars with low PUFA content. Particularly promising feeding strategies for a firm pork fat involve MCFA (Kreuzer et al., 1997) and CLA (see below). However, the first approach increases MCFA content of the pork at cost of unsaturated fatty acids which is highly undesired from a dietetic viewpoint, while the second approach might be advantageous also from health aspects.

Strategic improvement of fat shelf life in pork and poultry

Less prominent in the consumer's perception, but still highly relevant, is shelf life in pork (durable sausages) and poultry (e.g. instant soups). Shelf life is also dependent of PUFA content. Again, enrichment with dietetically desired unsaturated fatty acids is inappropriate, while low-fat diets are equivalent to MCFA-enriched diets in terms of fat shelf life (Kreuzer et al., 1997). Different from firmness, there exist other feeding strategies to improve shelf life from the feeding, in particular various antioxidants. Very effective in that respect is vitamin E where a basal level of 25 mg/kg should be increased by 1 mg/kg per g PUFA/kg feed in pig diets (Wenk, 1991). Vitamin-E supplementation is also improving the dietetic value of the pork, but no payment scheme considering extended shelf life and high vitamin E content exists so far.

Pasture beef

At present, in Europe there are contrasting trends concerning grazing or year-round silage feeding, the latter with the predominant use of maize silage. In terms of fatty acid profile of the beef, grazing is preferable to maize silage feeding although both feeds contain polyunsaturated fatty acids. However, grass mainly contains ALA which can be elongated and further desaturated to the long-chain ω -3 polyunsaturated fatty acids (LC ω -3PUFA) EPA, DPA and DHA (Sprecher, 2000). There is clear evidence for an enhanced proportion of ω -3 fatty acids in beef from grass (and linseed) fed bulls compared with beef from bulls fed maize silage and concentrate (Dannenberger et al., 2004). Retail beef with stated origin from grazing was found clearly superior in proportions of ω -3 fatty acids to conventional beef, too (Razminowicz et al., 2004). Regarding recommended dietary allowances, which range from 135 to 650 mg/day (Meyer et al., 2003), an additional intake of about 20 mg LC ω -3PUFA from 100 g of pasture beef and linseed-based beef vs. conventional beef seems small, particularly in relation to the actual disappearance of retail beef, which is clearly below 100 g per day in Western countries (about 82 g per capita and day in the USA and 30 g in Europe-15). However, the average fat content and, therefore, also the content of LC ω -3PUFA in the actually consumed beef is likely to be higher than in the pure muscle tissue. This is underlined by the findings that the consumption of animal products, which had been produced by supplementing extruded linseed in farm animal's diet, markedly increased the ω -3 fatty acids in the blood of volunteers (Weill et al., 2002). This illustrates that linseed might be a valuable source of ALA, too (although being obviously less effective than alpine grazing when milk shall be enriched with ω -3 fatty acids; Hauswirth et al., 2004). Either ALA or mono-unsaturated trans fatty acids may also be transformed into CLA, mainly into the *cis*-9,*trans*-11-C18:2 isomer (also called rumenic acid) as being the predominant and desired isomer naturally occurring in ruminant products (Kramer et al., 1997; Raes et al., 2004). This isomer mainly a product of endogenous desaturation of *trans*-vaccenic acid (from 'vacca' = cow), the predominant 18:1-*trans* isomer, in grass-fed cattle (Dannenberger et al., 2004). Accordingly, Chin et al. (1992) claimed that the best dietary sources of CLA are foods produced by grass-fed ruminants. A disadvantage of pasture beef might be the risk of the so-called grassy off-flavour (Melton, 1990) which mainly is an impression of rancidity resulting from the high proportion of LC ω -3PUFA of such beef.

Alpine milk and cheese

Looking at more extensive production systems, recently the particular dietetic value of alpine milk and cheese got increasing attention. Comparing lowland and alpine cheese, Innocente et al. (2002), Kraft et al. (2003) and Hauswirth et al. (2004) analyzed clearly higher contents of ω -3 fatty acids and CLA in alpine vs lowland cheese. Leiber et al. (2005), applying a specific experimental design, attempted to identify the factors being responsible for this effect. As outlined in the previous chapter, grass feeding vs. feeding mainly based on maize silage and concentrate alone is likely to increase the proportion of the desired fatty acids. Accordingly, there was no extra effect of alpine grazing on CLA in milk, while ALA, despite somewhat lower contents in alpine grass, was specifically elevated in proportion in alpine milk. Reasons for that could be plant secondary compounds reducing the hydrogenating activity of the rumen microbes or selective mobilization of ALA from body stores.

Strategic supplementation of functional fatty acids, or fats specifically rich in these fatty acids, to improve fats in animal-derived products

Ω -3 fatty acids

A large number of experiments has been carried out since the eighties to enrich ω -3 fatty acids in eggs, meat and milk. Most attempts are based on marine fish oil and linseed (Raes et al., 2004a), the first being rich in EPA and DHA and the latter having ALA as the prevalent ω -3 fatty acid. Microalgae could be an alternative source of DHA (Boeckart et al., 2005), provided the price is getting lower (actually algae are the source of the LC ω -3PUFA in marine animal species, too). It is known that ALA is less effective in the prevention of coronary heart diseases than LC ω -3PUFA but, as described above, can be transformed to some extent into these fatty acids in the body of mammals (this twofold: first in livestock and then in man consuming the product). Due to the prevalent risk of oxidation and, consequently, of off-flavours, ω -3 fatty acids are best enriched in products where these fatty acids are not exposed to oxygen during storage. Eggs are one option for that, and Farrell's ω -3 eggs (Farrell and Gibson, 1991) are famous since that approach was published (actually such eggs were first commercialized in Japan as early as in 1985 by the Nishin Flour Milling). In countries with large fish industries, such as Thailand, fish oil is a feed component with a reasonable price and therefore there are also various attempts to fortify pork with LC ω -3PUFA. Supplementing the same amount of tuna oil turned out to be similarly effective when provided either throughout fattening or only at the end

of fattening, while LC ω -3PUFA stored in the body of the pigs in the starter period seem to get lost to a considerable proportion once supplementation gets terminated in the finisher period (Table 1).

Table 1. Total ω -3 fatty acid proportion (% of total fatty acids) in pig muscle (*M. longissimus dorsi*) and backfat after feeding the same amount of tuna oil at different stages of fattening (Khiaosa-ard et al., 2005)*

Tuna oil, % of diet	–	1	3	3
Live-weight range, kg	–	30-100	80-100	30-60
Muscle tissue	1.6 ^b	2.1 ^a	2.1 ^a	1.7 ^b
Fat tissue	1.5 ^c	2.2 ^a	2.2 ^a	1.8 ^b

*Means carrying no common superscript are significantly different.

Conjugated linoleic acids

New attempts show that it is possible to enrich products of animal origin such as eggs (Schäfer et al., 2001; Tab. 2), pork (Scheeder et al., 2003), beef and lamb (reviewed by Raes et al., 2004a) with CLA through supplementation. However, the commercially available products for feeding are typically mixtures of desired and less desired isomers. A side-effect of CLA feeding is its pronounced effect on texture of the products. When avoiding extremes (which would provoke rejection by the consumers for visual appearance reasons; Schäfer et al.; 2001) and provided purified

Table 2. Effect of CLA (1:1 mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers) supplemented to laying hens when replacing linoleic acid (Schäfer et al., 2001)

Supplementation, 2 % of diet	Linoleic acid		CLA
CLA in yolk, g/100 g fatty acids	0.05	*	7.95
Oleic acid in yolk, g/100 g fatty acids	35.13	*	18.33
Yolk height/yolk width	0.46	*	0.52

* = significantly different ($P < 0.05$)

CLA isomers get available, this strategy could contribute to both firmer products and dietetically desired enrichments of CLA. However, it has to be kept in mind that a corresponding decrease in oleic acid takes place as the $\Delta 9$ -desaturase process is inhibited by CLA supplementation (Schäfer et al., 2001; Scheeder et al., 2003).

Phytanic acid

Phytanic acid is formed from phytol of plant origin (a side-chain of chlorophyll which can be cleaved from the complete molecule by the rumen microbes) and therefore ruminant meat and milk are naturally enriched with phytanic acid (Verhoeven and Jakobs, 2001). In monogastrics, phytanic acid may be enriched by feeding. Raes et al. (2004b) and Scheeder et al. (2005) demonstrated this for pork. Keeping in mind the uncertainties of the valuation of phytanic acid in a human health sense and the known severe risk of intake of phytanic acid for patients with disorder in α -oxidation metabolism such as Refsum's disease (Mukherji et al., 2003), a recommendation of its fortification in food of animal origin still seems premature.

The persistent contradiction in the goals and possible solutions from a more holistic approach

It has to be noted that even strategic approaches adding only a single fatty acid often disturbs the fragile equilibrium of maintaining a favorable fatty acid profile since other fatty acids are modified, too, be it only to allow the supplemented fatty acid to be incorporated. One example, where a more severe modification is resulting, is feeding (long-chain) polyunsaturated fatty acids to ruminants which does not only increase these fatty acids and CLA but also C18:1 *trans* fatty acids in the products. This also decreases the efficacy of enrichment of the desired fatty acid. Dohme et al. (2003) demonstrated that the capacity of the rumen for biohydrogenation seems limited, which suggests to use high rather than low amounts of e.g. of fish oil to ensure a high transformation rate to the products. Existing strategies include the MUFA : PUFA norm (Bee, 2004) in order to be able to cope with the requirements for a limited fat (iodine) number in Switzerland, payment systems for a favorable spreadability of the butter in winter, the recommendations of e.g. DACH (2000) concerning maximal and recommended intakes of certain fatty acids and ratios (for instance a ω -6 : ω -3 ratio in human diet of below 5:1). Again these approaches either aim either at a high dietetic or a high sensory/processing value of the products. Furthermore, payment

schemes for a particular dietetic quality are widely missing. Concepts which could be used for concerted efforts include the use linseed instead of sunflower seed or full-fat soy beans for improving butter spreadability in winter. The same is true for elevating the melting temperature of fat in beef and mutton. Also combining strategic fat supplementations with vitamin E additions is valuable for two goals simultaneously. These examples show that it is important to consider various rather than one strategic goal in order to maintain the fragile equilibrium between benefit and harm. This includes also the necessity to decide on the maximum supplementation levels which still do not result in severe dietetic, sensory or processing quality losses. Finally, production of eggs, milk and meat for specific markets (clinics, premium restaurants etc.) could be economically viable for innovative farmers.

Summary

This review describes the most important fatty acids with respect to human health value and sensory as well as processing properties of animal-derived products, and describes strategies successful in correspondingly improving the fat characteristics of the products. In detail, feeding for firm and stable pork fat, pasture beef, alpine milk and cheese as well as supplementation of ω -3 fatty acids, CLA and phytanic acid are described. The contradictory nature of many of such attempts is shown and it is suggested that the strategies are looked at in a more holistic view than done presently. In particular, where goals go into a similar direction like with good spreadability of butter in winter or beef and mutton fat with a desired high melting point, concerted efforts could be made with appropriate supplementary fats.

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Potentials of biotechnology to enrich valuable ingredients in crops under the special significance of vitamin E

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1. Modern biotechnology & classical plant breeding

Since the outset of agriculture plants have been genetically modified, but until recently this could only be achieved by the time-consuming crossbreeding of varieties. Furthermore, crossing is only possible within the species or between very closely related species. Genetic engineering is a new technique that promises to speed up this process and to expand the possible applications.

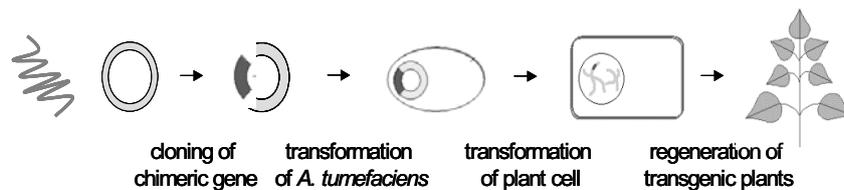


Figure 1. Generation of transgenic plants via *Agrobacterium tumefaciens* transformation.

Chimeric genes can be integrated into the plant genome via different transformation techniques thereby introducing new desirable characteristics from other varieties or adding new characteristics and functions from other unrelated species into the transgenic line (Fig. 1). These transgenic plants serve as origins for further use in traditional breeding of crops with modified qualitative and quantitative characteristics. After the green revolution most of the research on plant breeding and the potentials of biotechnology were used for projects aiming at the increase of crop yields by improving important traits such as insect, disease and herbicide resistance, modification of plant architecture and plant development as well as tolerance to abiotic stress. However, the recent applications of plant biotechnology to improve the nutritional content of food crops have perhaps the greatest potential to benefit global health. Examples of this sort of modification include the improvement of crop quality by expressing new proteins and enzymes that have a function as biopharmaceuticals

or which change the nutritional status of the crop and modify the macronutrient composition as well as micronutrients such as minerals and vitamins (Fig. 2).

- Increase and stabilize **crop yield** by

 - Improving important traits such as insect, disease and herbicide resistance
 - "Bt crops" expressing the delta-endotoxin gene from *Bacillus thuringiensis* a bacterium pathogenic for a number of insect pests
 - Transgenic tomato plants resistant against tobacco mosaic virus
 - Transgenic crop plants resistant to some of the newer herbicides (e.g., 2,4-D and bromoxynil)
 - Modification of plant architecture and plant development
 - e.g., height, early or late flowering or seed production
 - Tolerance to abiotic stresses
 - Salt-resistant tomatoes expressing a sodium/proton antiport pump
 - Transgenic *Arabidopsis* plants protected from dehydration and low temperature stress.

Enhance **crop quality** by

 - Production of biopharmaceuticals
 - Cavity-fighting apples inhibiting decay-causing bacteria
 - Transgenic bananas conferring resistance to hepatitis B
 - Transgenic plants expression genes for proteins to be used in medicine
 - Modifying the composition of macronutrients
 - High fructan beets
 - Protein enhanced maize, rice and potatoes supplying essential amino acids
 - High omega-3 vegetable oils decreasing cardiovascular disease risks
 - Enriching foods with minerals and vitamins
 - Iron-pumping rice
 - High vitamin E, C and provitamin A crops.

Figure 2. Examples of important biotechnology projects to improve crop yield and quality [1].

2. Engineering the vitamin content in transgenic plants

In the last years significant progress has been made in the molecular understanding of many vitamin related biosynthetic pathways and in the use of cloned genes to engineer plant metabolism. However, the full array of vitamin functions is not yet known and novel putative properties are being attributed to these molecules. Also, the biosynthetic pathways of vitamins in plants have not been fully elucidated and the regulation of vitamin biosynthesis is largely unknown. Once the pathways are known and the corresponding genes have been cloned, it is possible to engineer synthetic

pathways for vitamins in important crop plants and to shift levels of vitamins in order to create functional food with enhanced health benefits as well as extractable independent food additives. At present most satisfactory results have been obtained with transgenic plants expressing elevated levels of vitamin E, provitamin A and vitamin C. The careful examination of these projects highlights the potentials but also the currently anticipated limitations.

2.1. Engineering the vitamin E content in transgenic plants

Tocochromanols are lipid-soluble antioxidants collectively known as vitamin E and comprise a group of eight related isoforms [2].

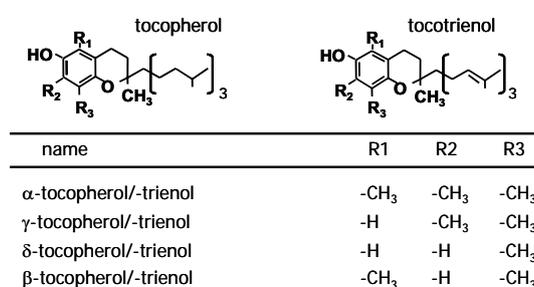


Figure 3. Tocochromanol structure and nomenclature.

The tocopherols consist of a polar chromanol ring with a saturated C16 prenyl side chain and comprise four homologous forms, namely α -, β -, γ - and δ -tocopherol, differing only in the number and position of methyl substituents on the chromanol head group, whereas the tocotrienols possess an unsaturated C16 prenyl group but otherwise the same substitution pattern on the chromanol ring (Fig. 3). These different tocochromanol forms have varying antioxidative abilities in scavenging oxygen radicals and quenching singlet oxygen, with the α -forms having the highest biological activity [2-4]. In addition tocochromanols appear to play a role in the preservation of membrane integrity by forming complexes with products of membrane lipid hydrolysis as well as in the regulation of transcription and post-translational processes [5-9].

Tocopherols are only synthesised in oxygenic photosynthetic organisms like cyanobacteria and higher plants. The biosynthesis of the different prenylquinones proceeds in plants at the inner envelope membrane of chloroplasts [10,11] and

requires the flux of intermediates from the isoprenoid as well as the aromatic compound synthesis.

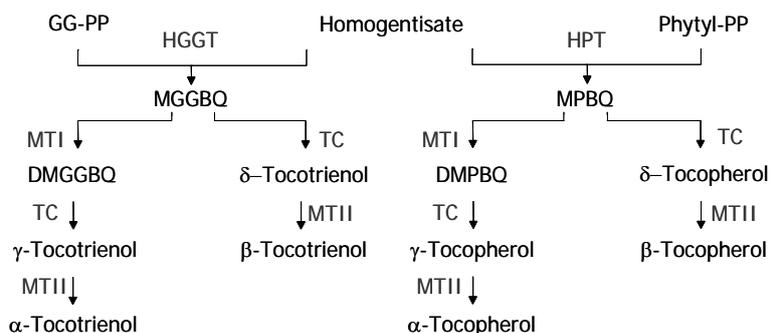


Figure 4. Schematic representation of tocopherol biosynthesis in plants. HGGT, homogentisate geranylgeranyltransferase; HPT, homogentisate phytyltransferase; MTI, MPBQ/MGGBQ methyltransferase; TC, tocopherol cyclase; MTII, γ -tocopherol methyltransferase; MGGBQ, 2-methyl-6-geranylgeranyl-1,4-benzoquinol; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinol; DMGGBQ, 2,3-dimethyl-5-geranylgeranyl-1,4-benzoquinol; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinol.

As shown in Fig. 4. the committed step in this pathway is catalysed by a membrane bound prenyltransferase transferring a prenyl group from phytyl pyrophosphate (phytyl-PP) or geranylgeranyl pyrophosphate (GG-PP) to homogentisate, so that 2-methyl-6-phytylplastoquinol (MPBQ) or 2-methyl-6-geranylgeranylplastoquinol (MGGBQ) are produced [12-17]. The chromanol head group is subsequently formed by the activity of a tocopherol cyclase [18,19] and two different methyltransferases are responsible for the methylation pattern at the chromanol ring. In plants the TC cyclises preferentially DMGGBQ and DMPBQ yielding the respective γ - , and after further methylation, α -tocopherols, whereas the δ - and β -tocopherols, the cyclisation and methylation products of MGGBQ and MPBQ, are only existent in marginal amounts [3,18,19].

The main features of the biosynthetic pathway of prenylquinones in plants have been elucidated several years ago using classical biochemical methods [20,21], but the genes encoding the respective enzymes of this pathway have only been cloned during the past few years [13,16,22-25]. Different approaches have been undertaken to manipulate vitamin E content and composition in plants and examples of

biotechnological projects aiming at the elevation of vitamin E content in the seed oil of transgenic plants are listed in Table 1.

Table 1. Maximal tocochromanol increases in the seed oil of transgenic plants.

Plant	Increase in tocochromanol content	Tocochromanol composition	Expressed Recombinant enzyme	Reference
<i>A. thaliana</i>	1.4	Toc	HPT	[26]
<i>A. thaliana</i>	1.8	Toc	HPT	[16]
<i>B. napus</i>	1.4	Toc	HPT	[27]
<i>B. napus</i>	1.5	Toc, P8	TC	
<i>B. napus</i>	1.4	Toc	HPPD	[28]
<i>B. napus</i>	2.4	Toc, P8	HPT, HPPD, TC	
<i>A. thaliana</i>	3.5	Toc, Tri	HPT, HPPD, HPPS	
<i>B. napus</i>	3.7	Toc, Tri	HPPD, HPT, HPPS	[29]
<i>A. thaliana</i>	5	Toc, Tri	HPPD, HPT, HPPS	
<i>Glycine max</i>	14.7	Toc, Tri	HPPD, HPPS, HPT, GGR	
<i>Zea mays</i>	4-6	Tri	HGGT	[30]

Overexpression of the homogentisate prenyltransferase catalysing the first committed step in tocopherol biosynthesis resulted in significant increases in the total tocochromanol content of up to 1.4 (Table 1). Likewise, the overexpression of a tocopherol cyclase (TC), another key enzyme of the pathway, led to a distinct rise in total tocochromanol content (Table 1). Furthermore, the analyses of the TC overexpressing plants revealed interesting changes in the overall tocochromanol pattern and gave new insights into the substrate specificity of the TC from *Arabidopsis thaliana*. This enzyme was able to utilise not only DMPBQ but also MPBQ and plastoquinone-9 (PQ9) for cyclisation, yielding higher levels of δ -toc and plastochromanol-8 (P8), respectively [27] (Fig. 5). The production of P8, a chromanol compound naturally occurring only in the seed oil of few plant species like *Brassica napus*, *Linum usitatissimum* and *Cannabis sativa* [31-34] demonstrated that the PQ9 and tocopherol biosynthetic pathways are closely connected with each other and that they share common enzymes.

The tocopherol methyltransferase I (MT I) is also active in both pathways and exerts, as demonstrated by Cheng et al. [35], a distinct influence on tocopherol content and composition in the leaves of *Arabidopsis* plants. Whereas the γ -tocopherol methyltransferase (MTII) bears no influence on the overall tocopherol content, but effects an increase in the vitamin E level by almost completely shifting γ -toc to α -toc as well as δ -toc to β -toc [36].

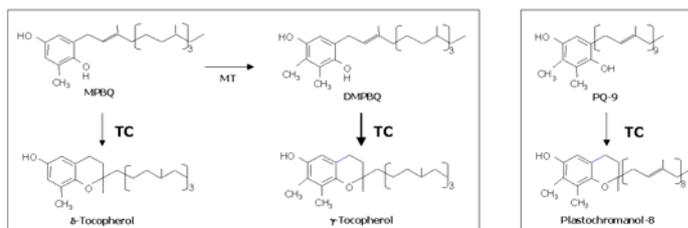


Figure 5. Chromanol headgroup formation catalysed by TC utilising MPBQ, DMPBQ and Plastoquinone-9 (PQ-9) as substrate [27].

According to these data, the overexpression of key enzymes from the tocopherol pathway has a significant impact on total tocochromanol content but is not sufficient to reach a several fold yield of tocopherols. So the analysis of the different transgenic plants underlined the experiences made in the elucidation of biosynthetic pathways by asserting that the metabolic flux in pathways is often not controlled by a single rate limiting enzyme [37,38]. In addition these analyses also gave strong indications that the channelling of precursors from the plastidial shikimate and DXP-pathways is of special importance (Fig. 6). This assumption was supported by the overexpression of 4-hydroxyphenylpyruvate dioxygenase genes (HPPD), providing the aromatic precursor homogentisate, alone and in combination with HPT and TC genes, effecting a significant 2.4-fold increase in total tocopherol content in the transgenic plants (Table 1). A further boost in precursor supply from the shikimate pathway by expression of a bifunctional hydroxyphenylpyruvate synthase (HPPS), thereby shortcutting some strong regulated steps in the shikimate pathway, lead to a further huge rise in total tocochromanol levels [28,29,39]. The boost in the plastidial homogentisate pool in transgenic plants resulted in the activation of an endogenous HGGT activity and, thus, in the accumulation of tocotrienols whereas the tocopherol content was hardly effected [29,40]. A concomitant boost in the plastidial phytylPP pool [29,41] in such transgenic plants will show whether the tocopherol content is primarily controlled by the substrate pools available to the HPT or the properties of the enzyme.

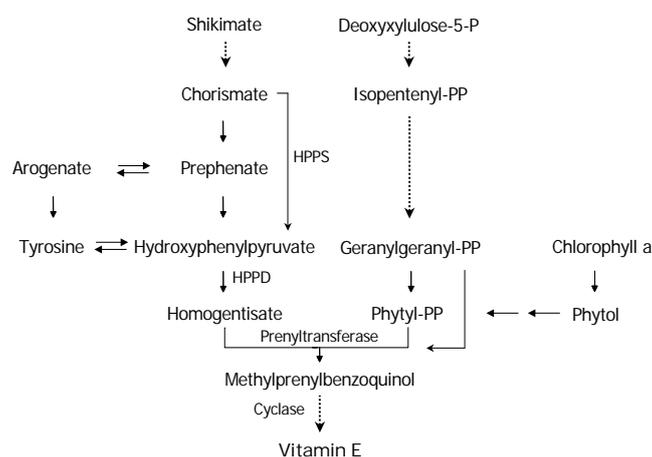


Figure 6. Supply of aromatic and isoprenoid precursors for vitamin E biosynthesis. HPPD, 4-hydroxyphenylpyruvate dioxygenase; HPPS, 4-hydroxyphenylpyruvate synthase.

Altogether the data obtained by the analysis of the tocochromanol biosynthesis point out that a several fold increase of the tocochromanol content in the seeds of transgenic plants requires an upregulation of the pathway by overexpressing multiple rate limiting enzymes as well as a reinforced supply of the aromatic and isoprenoid precursors. Hence, the analysis of the transgenic plants facilitated new insights into the complex mechanisms, which regulate the metabolic flux of substrates and intermediates in the tocochromanol pathway and represent new potent tools to study the underlying regulatory mechanisms and to reveal further target genes limiting to biosynthesis and accumulation of vitamin E in plants.

2.2. Engineering the provitamin A content in transgenic plants

Carotenoids, especially β -carotene (provitamin A) are important in the animal and human diet because they are essential precursors for the synthesis of the lipophilic vitamin A (retinol), which is of vital importance for the protection of vision, the function of the immune system as well as the reproduction and embryonic development of animals and humans [42].

Provitamin A biosynthesis starts with geranylgeranyl-PP, an intermediate in the plastidial isoprenoid metabolism (Fig. 7), but the end product β -carotene does not accumulate in equal amounts in all plant tissues.

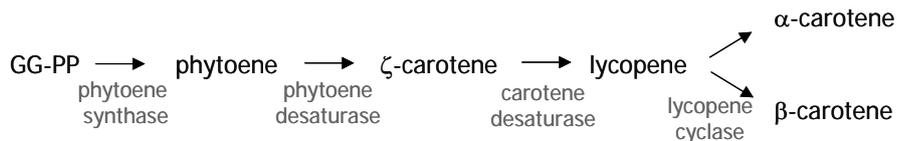


Figure 7. Schematic representation of provitamin A biosynthesis in plants.

Rice for instance is an important staple food in worldwide nourishment but contains no β -carotene in the endosperm, the edible part of the rice grain, thereby contributing to provitamin A malnutrition. In order to improve the nutritional value of rice grain several approaches to improve the β -carotene content in the endosperm have been undertaken during the last few years [39], and Ye et al. [43] succeeded in developing the so called “golden rice”, which contains up to 200 μg β -carotene per 100 g. But similar to the observations made in the vitamin E engineered plants, the introduction of heterologous genes had an influence on the regulation of isoprenoid metabolism and some positive and negative feedback mechanisms by intermediates on endogenous genes have been postulated [39]. This knowledge provided the basis for the recent development of a second generation of transgenic rice containing up to 23-fold more β -carotene in the grain than the original golden rice [44]. With these transgenic rice plants the provision of the recommended dietary allowance of vitamin A through alimentation with rice grain comes now into reach.

Finally, further successful examples for the manipulation of vitamin levels in transgenic plants have to be noted. Utilising different genetic approaches, Jain and Nessler [45] as well as Agius et al. [46] successfully engineered vitamin C biosynthesis in transgenic tobacco, lettuce and strawberry plants and obtained up to 4–7 fold increases in vitamin C content [45,46].

3. Conclusions

Plants have a great potential as production systems of vitamins for the future. Yet at the moment, a gap remains between the ability to clone and manipulate individual genes and the understanding of how they integrate in and impact on plant

metabolism. The analysis of the transgenic plants from the present generation facilitated new insights into the complex regulation mechanisms, which steer the metabolic flux of substrates and intermediates in pathways, and provided a promising basis for the elucidation of the underlying principles. The current results already show clearly the great potentials of plants as production systems for tailor-made functional food with increased levels of valuable compounds.

4. References

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Vitamin D metabolites: Physiological role in bone and pancreas and therapeutic potential for bone diseases and diabetes

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Introduction

Vitamin D from endogenous, cutaneous synthesis or from dietary sources is ineffective as such and has to be metabolically activated by two hydroxylation steps occurring in liver and kidney, yielding the vitamin D hormone $1\alpha,25$ -dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] or calcitriol. $1,25(OH)_2D_3$ is the major biologically active form of vitamin D, and acts through a nuclear receptor, the vitamin D receptor (VDR). The VDR belongs to the nuclear receptor superfamily (Evans, 1988), and regulates gene transcription by binding to vitamin D-responsive elements in the promoter region of target genes. The vitamin D hormone is essential for an intact mineral metabolism, and vitamin D deficiency or any block of the subsequent activation steps or of signaling through the VDR results in rickets or osteomalacia. Functional inactivation of the VDR by gene targeting in mice or by genetic defects in the VDR gene in humans results in rickets (vitamin D-dependent rickets type II, VDDRII) and alopecia.

VDR expression is not limited to organs involved in calcium homeostasis such as bone, kidney, duodenum, and parathyroid gland but has also been demonstrated in various other tissues (Haussler et al., 1998). In fact, VDR is expressed almost ubiquitously. Therefore, $1,25(OH)_2D_3$ may have important physiological functions beyond calcium homeostasis. For example, $1,25(OH)_2D_3$ regulates keratinocyte differentiation in the skin, has immunomodulatory functions, and may also be involved in pancreatic insulin secretion (Haussler et al., 1998).

Physiological functions of the vitamin D hormone in bone

One of the most important functions of $1,25(OH)_2D_3$ in the regulation of calcium homeostasis is the stimulation of intestinal absorption of calcium and phosphorus. However, it has not been known until recently whether $1,25(OH)_2D_3$ also has an essential function in bone. As osteoblastic cells (Narbaitz et al., 1983) and early precursor cells for osteoclasts (Roodman, 1996) express VDR, physiologically important

direct functions of $1,25(\text{OH})_2\text{D}_3$ in bone would be conceivable. In accordance with this idea, it is well established that $1,25(\text{OH})_2\text{D}_3$ can modulate osteoblast proliferation and osteoblast production of type I collagen, alkaline phosphatase, and osteocalcin *in vitro* (Reichel et al., 1989). However, some studies in vitamin D-deficient rats have questioned a direct role of the vitamin D hormone in bone. For example, bone mineralization is normal in vitamin D-deficient rats infused with adequate amounts of calcium and phosphorus (Weinstein et al., 1984), and vitamin D-deficient rats can be maintained on a so-called rescue diet for prolonged periods of time without any overt impairment of bone mineralization (Walters et al., 1992). The rescue diet is enriched with calcium and phosphorus, and contains a high amount of lactose which stimulates the non-specific intestinal uptake of calcium and phosphorus (Kollenkirchen et al., 1991).

The most compelling evidence showing that vitamin D metabolites do not have an essential function in bone comes from studies in VDR knockout mice. Importantly, calcium homeostasis, serum PTH, and all bone abnormalities in VDR deficient mice can be corrected by feeding the abovementioned rescue diet, starting a few days before weaning (Li et al., 1998; Erben et al., 2002b). Therefore, the sole function of the vitamin D endocrine system for bone is to provide adequate amounts of calcium and phosphorus for bone mineralization through stimulation of their intestinal absorption. $1,25(\text{OH})_2\text{D}_3$ does not have physiologically important direct functions in bone. Thus, all the skeletal effects of vitamin D deficiency are mediated indirectly through decreased intestinal mineral absorption and the accompanying secondary hyperparathyroidism.

Pharmacological effects of vitamin D analogs on bone

Although the endogenous vitamin D hormone does not appear to have an essential function in bone, pharmacological administration of vitamin D analogs induces profound changes in bone metabolism that are very likely mediated through a local action on bone cells.

It is well established that chronic administration of active vitamin D metabolites to rats under the conditions of a sufficient dietary calcium intake results in a dose-dependent suppression of bone resorption (Erben et al., 1992; Wronski et al., 1986; Erben et al., 1998b; Erben et al., 1998a; Shiraishi et al., 2000; Weber et al., 2001). The same is

true for humans (Need et al., 1985). The main mechanism for the antiresorptive effect of chronic vitamin D analogs *in vivo* is probably PTH suppression.

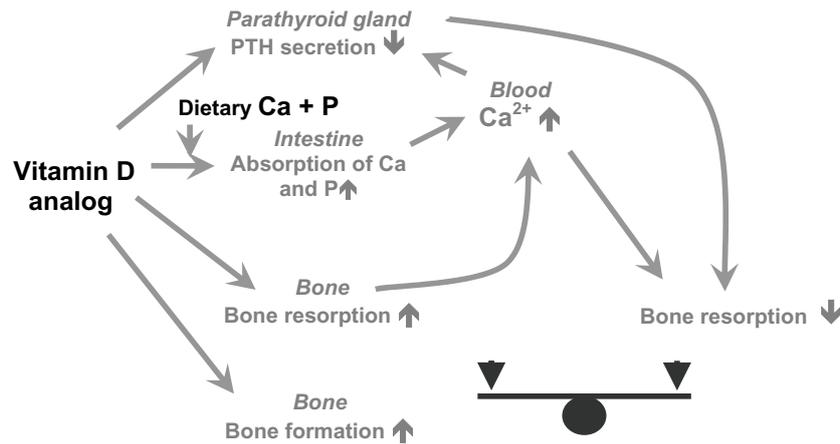


Fig. 1. Effects of pharmacological treatment with vitamin D analogs on bone. External administration of an active vitamin D analog lowers PTH secretion mainly indirectly via stimulation of intestinal calcium absorption and a subsequent rise in serum calcium, and by a direct inhibitory activity on the parathyroid gland at the transcriptional level. PTH suppression down-regulates bone turnover, i.e., bone resorption and bone formation. At higher dosages, bone formation is stimulated through a direct action on bone-forming cells. This effect overrides the suppression of bone formation by diminished PTH serum levels. Vitamin D analogs may also have a direct stimulating effect on bone resorption. However, the latter effect may be of relevance only for short-term administration of high doses of vitamin D analogs. In addition, some experiments have suggested a direct antiresorptive effect of vitamin D analogs. The role of the latter effect is unclear at present.

This notion is corroborated by the finding that the suppressive effect of 1,25(OH)₂D₃ on osteoclast number in ovariectomized (OVX) rats is modulated by dietary calcium (Erben et al., 1998b), that 1,25(OH)₂D₃ treatment fails to suppress osteoclast number in intact rats on a low-calcium diet (Boyce and Weisbrode, 1983), and that the antiresorptive action of 1,25(OH)₂D₃ is modulated by the route of administration (Erben et al., unpublished results). However, experiments in parathyroidectomized rats infused over 2 weeks with human PTH-related peptide(1-34) and simultaneously orally treated with 1,25(OH)₂D₃ or 22-oxacalcitriol showed that vitamin D analogs are able to dose-dependently reduce serum calcium in this model of humoral hypercalcemia of malignancy, suggesting a direct antiresorptive effect on bone (Endo et al., 2000).

Recent experiments conducted in our own laboratory also suggested that 1,25-(OH)₂D₃ had antiresorptive effects in parathyroidectomized rats constantly infused with rat PTH (Erben et al., unpublished results). However, the antiresorptive effect in the latter experiment occurred in the presence of hypercalcemia. Therefore, the available evidence suggests that such a direct antiresorptive pathway is of minor importance for chronic treatment with vitamin D analogs under normal circumstances (Fig. 1).

Due to their antiresorptive effect, low oral doses of vitamin D analogs are able to prevent cancellous and cortical bone loss induced by estrogen deficiency (Erben et al., 1992; Erben et al., 1998a; Shiraishi et al., 2000) or immobilization (Okumura et al., 1990) in rats, and correct cancellous bone loss in ovariectomized dogs (Malluche et al., 1988).

One of the greatest challenges in the therapy of osteoporosis is to find a cost-effective and safe bone anabolic treatment that would effectively increase bone tissue in an osteopenic skeleton. Among the other major substance classes that have been shown to possess bone anabolic properties *in vivo*, namely intermittent parathyroid hormone (PTH), prostaglandins, fibroblast growth factors, and growth hormone, vitamin D analogs have the advantage of being orally available.

A number of experimental studies in rats have clearly shown that vitamin D analogs are not only able to prevent estrogen deficiency-induced bone loss by an antiresorptive mechanism but can have pronounced bone anabolic effects at higher dosages (Wronski et al., 1986; Erben et al., 1997; Erben et al., 1998b; Weber et al., 2001). When higher doses of vitamin D analogs are administered to normal or osteopenic rats, cancellous bone mass in nongrowing or slowly growing bone sites increases above the level seen in baseline controls or in sham-operated control animals (Erben et al., 1997; Erben et al., 1998b; Shiraishi et al., 2000). It is known that purely antiresorptive drugs such as bisphosphonates do not increase cancellous bone volume in nongrowing bone sites (Qi et al., 1995). Therefore, the antiresorptive activity cannot account for the bone anabolic effect induced by vitamin D analogs in nongrowing bone sites. Rather, such an effect must involve a net increase of bone formation over bone resorption. Recent studies in OVX rats have shown that high doses of vitamin D analogs increase the total cross-sectional area of the tibial shaft (Weber et al., 2001; Weber et al., 2004). Therefore, vitamin D analogs are not only anabolic for cancellous but also for cortical bone through stimulation of periosteal bone apposition. The

increase in cancellous and cortical bone mass induced by $1,25(\text{OH})_2\text{D}_3$ in animal studies is paralleled by similar increases in biomechanical bone strength (Erben et al., 2002a). Therefore, the increase in bone mass induced by vitamin D analogs is associated with an improvement in bone quality.

An important question is whether the bone anabolic actions of vitamin D analogs are direct or indirect. The available experimental data support the notion that the increase in cancellous bone mass induced by chronic treatment with vitamin D analogs is based on a direct, pharmacological effect on bone (Erben et al., 1998b).

In contrast to their clear efficacy in animal studies, vitamin D analogs have not shown uniformly positive results in clinical osteoporosis trials. The wide use of vitamin D metabolites in osteoporotic patients, especially at higher doses, has been hampered by the major side effects of this compound class, hypercalcemia and hypercalciuria, due to the excessive stimulation of intestinal calcium absorption. Although several large studies have suggested that active vitamin D analogs increase BMD in the spine and forearm, and reduce vertebral fractures in osteoporotic patients (Gallagher and Goldgar, 1990; Tilyard et al., 1992; Shiraki et al., 1993), other studies have provided negative results (Ott and Chesnut, 1989). Therefore, the role of vitamin D metabolites in the treatment of osteoporosis is still controversial. Nevertheless, the clear potential for bone anabolic properties together with oral availability have stimulated the interest in this substance class.

Physiological effect of the vitamin D hormone in the endocrine pancreas

Early *ex vivo* studies by Norman and coworkers (Norman et al., 1980) have shown that insulin but not glucagon release after stimulation with glucose and arginine is reduced in the isolated perfused pancreas from vitamin D-deficient rats. Later on, the same group showed that glucose tolerance and insulin secretion are impaired in vitamin D-deficient rats *in vivo* (Cade and Norman, 1986), and that insulin secretion was improved within 3 hours after a single administration of $1,25(\text{OH})_2\text{D}_3$ to vitamin D-deficient rats (Cade and Norman, 1987). More recent studies reported that *de novo* insulin synthesis is reduced in isolated islets from vitamin D-deficient rats, and that insulin biosynthetic capacity can be restored *in vitro* by addition of $1,25(\text{OH})_2\text{D}_3$ (Bourlon et al., 1999). A few small clinical studies also suggested a role of vitamin D in insulin secretion. For example, vitamin D supplementation to patients at-risk of

diabetes improved the insulin secretory response after an oral glucose challenge (Boucher et al., 1995), and calcitriol treatment augmented insulin secretion and improved glucose tolerance in uremic patients (Allegra et al., 1994).

Additional evidence linking the VDR to the endocrine functions of the pancreas came from epidemiological studies showing that VDR restriction site polymorphisms are associated with the genetic susceptibility to type 1 diabetes in different populations (McDermott et al., 1997; Chang et al., 2000; Pani et al., 2000), and that vitamin D supplementation in early childhood is associated with a reduced risk for type 1 diabetes (The EURODIAB Substudy 2 Study Group, 1999; Hypponen et al., 2001). It is not clear, however, whether the linkage of VDR with type 1 diabetes susceptibility may be due to the immunomodulatory effects of the vitamin D hormone.

Recently, we have shown that disruption of the VDR signaling pathway is associated with a pronounced impairment in oral glucose tolerance and insulin secretory capacity, together with a reduction in pancreatic insulin mRNA levels in VDR mutant mice (Zeitz et al., 2003). These changes were independent of alterations in body weight or mineral homeostasis, suggesting a molecular role of the VDR in the endocrine function of the pancreas *in vivo*. In addition, we found a similar impairment in both oral and subcutaneous glucose tolerance in VDR mutants, suggesting that alterations in the enteroinsular axis are not involved in the diminished insulin secretory response in mice with a nonfunctioning VDR. This idea is corroborated further by earlier reports of reduced insulin secretion in the isolated perfused pancreas from vitamin D-deficient rats (Norman et al., 1980), and also by the finding that the insulin biosynthetic capacity can be restored in isolated islets from vitamin D-deficient rats *in vitro* by addition of 1,25(OH)₂D₃ (Bourlon et al., 1999).

VDR mutant mice demonstrated normal pancreatic β cell mass, normal architecture of pancreas islets, and a normal number of small clusters of insulin-producing cells (Zeitz et al., 2003). These findings suggest that fetal and postnatal islet development as well as the intensity of islet neogenesis during postnatal life are unchanged in VDR mutant mice. Therefore, the reduced insulin secretory capacity in VDR mutants is not based on a developmental or structural defect but is rather caused by functional alterations within insulin-producing cells. Because mice with a nonfunctioning VDR had normal fasting blood glucose and insulin levels, the defect in pancreatic insulin secretion is latent and is seen only when the mice are challenged with glucose. However, disturbances in the vitamin D signaling pathway may compromise

the β cell's ability to functionally respond to situations of an increased insulin demand such as in type 2 diabetes or in the prediabetic phase of type 1 diabetes.

The impaired insulin secretion in VDR mutants may be caused by a reduction in the amount of insulin stored in β cells. This notion is indirectly corroborated by the finding that $1,25(\text{OH})_2\text{D}_3$ does not enhance insulin secretion from islets of vitamin D-replete rats or of rat insulinoma cells (Lee et al., 1994). The stimulatory effect of $1,25(\text{OH})_2\text{D}_3$ on islet cell insulin synthesis (Bourlon et al., 1999) may, by direct or indirect pathways, involve increased transcriptional activity of the insulin gene or increased insulin mRNA stability. So far, no vitamin D response elements were reported in the human or mouse insulin gene promoters.

In addition, recent evidence has suggested that peripheral insulin sensitivity is strongly influenced by vitamin D status in normal individuals from different ethnic groups in California (Chiu et al., 2004). It is interesting in this context that vitamin D response elements were found in the human insulin receptor gene promoter, suggesting that the insulin receptor may be under transcriptional control of the vitamin D hormone (Maestro et al., 2003). Thus, non-calcemic vitamin D analogs may have a potential for the pharmacological stimulation of insulin secretion and for augmenting insulin sensitivity in the future.

Summary

It is well established that the vitamin D hormone $1\alpha,25$ -dihydroxyvitamin D_3 has an essential role for mineral metabolism. Vitamin D analogs act through the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily. Research conducted within the last years has conclusively shown that vitamin D metabolites lack an essential physiological function in bone, and that the development of rickets in the course of vitamin D deficiency is solely caused by impaired intestinal calcium absorption and subsequent secondary hyperparathyroidism. It is, on the other hand, well established that pharmacological administration of vitamin D analogs can have pronounced bone anabolic effects, and there is good evidence from a variety of studies that at least some of the bone anabolic properties of vitamin D analogs are independent of the untoward calcemic effects. In addition, recent experiments have suggested that the suppression of bone resorption seen under treatment with vitamin D analogs depends on the route of administration, and is mediated both by a direct antiresorptive effect, and by indirect effects via hypercalcemia and suppression of parathyroid hormone secretion. Apart from its essential role in mineral metabolism, the

vitamin D hormone may have important functions in other organ systems. For example, it is well known that pancreatic islets show strong expression of the VDR. We have recently shown that gene-targeted mice with a functionally inactive mutant VDR show impaired oral glucose tolerance and reduced insulin secretory capacity, independent of changes in calcium homeostasis. Additional evidence linking the VDR to the endocrine functions of the pancreas came from epidemiological studies showing that VDR restriction site polymorphisms are associated with the genetic susceptibility to type 1 diabetes in different populations, and that vitamin D supplementation in early childhood is associated with a reduced risk for type 1 diabetes. In addition, recent studies have indicated that peripheral insulin sensitivity may be regulated by the vitamin D status. Therefore, vitamin D analogs with reduced calcemic activity may have a future role in the prevention and therapy of diabetes.

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On the mode of action of micro-organisms

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This contribution reports preliminary results of an interdisciplinary project and includes contributions from five institutes of the Veterinary Faculty:

Institute of Animal Nutrition: Macha, M., Taras D., Vahjen, W.

Institute of Veterinary Anatomy: Reiter, K., Weyrauch K.D.

Institute of Veterinary Physiology: Lodemann, U., Lorenz, B., Martens, H.

Institute of Microbiology and Epizootics: Kleta, S., Nordhoff, M., Pollmann, M., Schierack, P., Schwerk, P., Tedin, K., Wieler, L.

Institute of Immunology and Molecular Biology: Altherr, B., Scharek, L., Schmidt, M.F.G.

Introduction

The use of micro-organisms in human food has a long history and goes back to Elie Metschnikoff. He proposed almost 100 years ago the hypothesis that bacteria in fermented milk products may be capable of controlling microbial fermentation in the intestine and prevent arteriosclerosis. Probiotics are defined in human nutrition as viable micro-organisms which show health promoting effects at sufficient oral intake rates. Especially during the last decade, the concept of probiotics has been applied in animal nutrition as well. The definition of probiotics in animal nutrition differs slightly from that applied in human nutrition and refers to viable forms of micro-organisms which are applied as feed additives and should lead to beneficial effects for the host animal due to an improvement of the intestinal microbial balance or properties of the indigenous micro-flora. In nutrition of farm animals, beneficial effects are mainly expressed in terms of stimulated weight gain, improved feed conversion ratio and especially in pigs reductions in the incidence of post-weaning diarrhoea.

With regard to performance in pig and poultry nutrition, the published data indicate a trend towards promoting effects of probiotics, however, the improvements were only rarely significant. On the other hand, published data on the incidence of diarrhoea indicate in approximately 80 % of the experiments a significant reduction in piglets receiving probiotics.

The description on the mode of action of probiotics within the definitions shows that there is still no hard data to precisely explain probiotic effects.

Preliminary results of an interdisciplinary study on the mode of action of probiotics in pigs

Goal and experimental set up

Four years ago an interdisciplinary research group of the German Research Foundation (DFG) was established at the Freie Universität Berlin with the aim of obtaining more precise information about the complex effects of probiotic micro-organisms in the intestinal tract and the interaction between the intestinal microbiota and the host animal. The research project "Integrative analysis of the mode of action of probiotics in pigs" is supported by the DFG under the code (FOR 438) and included in the first phase the institutes of Animal Nutrition, Anatomy, Physiology, Microbiology and Immunology of our faculty.

First, we had to decide which probiotics should be included in the study. Out of 21 probiotic preparations actually authorised for the use as feed additives in the EU, 13 are approved for use in piglets and only a few for use in feed for sows and fattening pigs. Seven of these preparations include selected strains of *Enterococcus faecium* (natural habitat: digestive tract), two contain spores of the bacterial genus *Bacillus* (natural habitat: soil), two are strains of the *Saccharomyces cerevisiae* yeast (natural habitat: fruits), and one product contains *Lactobacillus farciminis* or *Pediococcus acidilactici*, for which the natural habitat is the digestive tract and milk products. It was decided to study one probiotic micro-organism originally isolated from the intestinal tract (*E. faecium* NCIMB 10415) and one spore-forming micro-organism originating from soil (*B. cereus* var. *toyo* NCIMB 40112).

In two experimental series these probiotics were fed to sows during gestation and the 28-day lactation period, to suckling piglets with the prestarter diet from day 15 onwards and after weaning up to day 56 as a supplement of the starter diet. Corresponding control animals received the same diets without the probiotic and were housed completely separate from the treated animals. For each treatment at least 10 sows and the resulting piglets (not less than 9 per litter) were studied. For recording the various parameters in order to study the mode of action of the applied micro-organisms, 5 piglets of both control and probiotic groups were sacrificed on days 14 (before receiving prestarter), 28 (at weaning), 35 (one week after weaning) and 56 (four weeks after weaning). All parameters were measured in samples originating from the same animals.

Recorded parameters (not all listed):

Morphology/histology of the intestinal tissue at different seats: villi shape, villi length, crypt depth, cell proliferation, rate of apoptosis, proportion of goblet cells, alkaline and acidic phosphatase.

Physiological parameters of the intestinal tissue: transcellular (glucose, glutamine) and paracellular (mannitol) transport rates, stimulation kinetics.

Intestinal microbiota: Specific enumeration of the probiotic strains, similarity of microbiota, total anaerobic and coliform bacteria loads, isolation frequency of β -haemolytic and selected *E. coli* serogroups, presence of *Chlamydiaceae*.

Immunological parameters: IgG and IgA concentrations in blood and faeces, isolation and detection of lymphocyte cell populations from the proximal jejunal epithelium and continuous Peyers patches.

Influence of the probiotics on post-weaning diarrhoea

Diarrhoea is the main problem for piglets during the first few weeks after weaning, consequently reduction of the incidence of diarrhoea by probiotics would be of major importance for the producer. In our study, faecal consistency was estimated daily using a macroscopic score from one to five (firm to watery). The macroscopic assessment of consistency score was validated by the dry matter content of faecal aliquots in lyophilized samples.

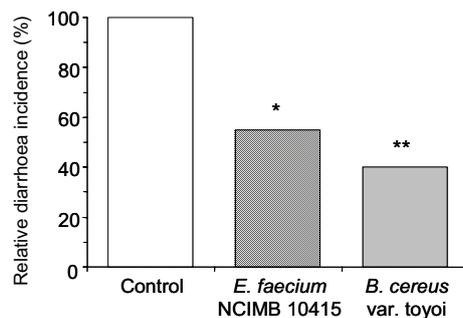


Figure 1: Relative diarrhoea incidence (Control = 100%) in weaned piglets receiving diets supplemented with *E. faecium* NCIMB 10415 and *B. cereus* var. toyoi, respectively. *, $p < 0.05$ and **, $p < 0.01$ compared to incidence in the control group

With both micro-organisms (*E. faecium* NCIMB 10415 and *B. cereus toyoi*) studied, a significant reduction in the incidence of diarrhoea in comparison to control animals was recorded (Figure 1). Compared to the control animals, the incidence of diarrhoea

was only 55 and 40 % in piglets when *E. faecium* or *Bacillus* probiotics were included in the diet, respectively. Performance in terms of daily weight gain and feed conversion was not influenced significantly by the *E. faecium* probiotic, but overall feed efficiency was significantly improved during the first four weeks after weaning in piglets receiving diets supplemented with the *Bacillus* probiotic. An additional factor resulting from the reduced incidence of diarrhoea would be a reduction in required veterinary interventions, which may be cost-saving for the producer.

Effects on the intestinal microbiota

Because probiotics are defined as viable forms of micro-organisms for which the beneficial effects stem at least in part from their metabolic activity in the intestinal tract, a key question is whether or not the applied micro-organism is able to pass the stomach barrier and to colonize the intestinal tract. Therefore, methods for specific enumeration of both applied bacterial strains were established. In the case of *B. cereus*, this was accomplished using conventional microbiological methods. For the *E. faecium* probiotic, a strain-specific probe for colony hybridization was constructed based on specific nucleotide sequences (Macha et al., 2004). For both probiotics, an earlier observation made with the *Bacillus* strain (Jadamus et al., 2001) was confirmed: that the probiotic bacteria are transferred from the sow to the suckling piglet most likely by contact with maternal faeces before prestarter feed was available, reaching cell numbers in the range of 10^4 to 10^5 cfu/g digesta in jejunum and colon. The concentration of the *E. faecium* probiotic was not considerably increased after intake of supplemented feed (10^6 cfu/g feed), whereas the concentration of the *B. cereus* probiotic increased by one order of magnitude after weaning (Taras et al., 2005; Figure 2). Neither of the probiotic strains were found to be present in control animals.

Considering that the use of micro-organisms of different origins, habitats and metabolic activities still show similar beneficial effects, it might seem logical that their modes of action will probably be based on more than one principle. However, the modification of the microbial population seems to be the prime mode of action. The interaction between the probiotic strain and the intestinal microbiota may be based on aggregation with pathogenic bacteria, competitive adhesion to epithelial receptors, production of specific substances (organic acids, bacteriocins, dipicolinic acid), or competition for nutrients.

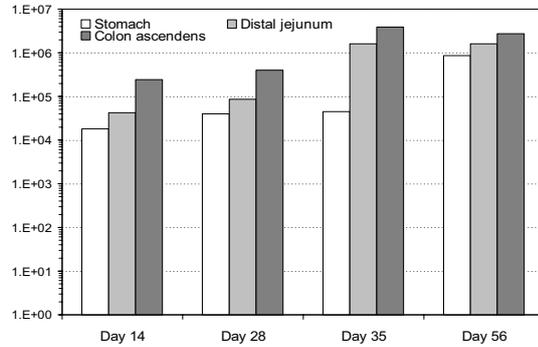


Figure 2: Total viable counts [cfu/g DM] of *Bacillus cereus* var. *toyoi* in digesta of piglets

In order to follow changes or general modifications of the dominant intestinal microbiota in our studies, denaturing gradient gel electrophoresis (DGGE) and construction of similarity indices based on DNA extracts were applied. It was observed that banding patterns from probiotic-fed piglets (*E. faecium*) clustered in a similar group, whereas animals fed a not supplemented diet exhibited a more diverse population. It was concluded that the bacterial community of probiotic-treated piglets was modified and less diverse compared to control animals. Preliminary results from the DGGE analyses of DNA extracts from animals receiving the *B. cereus* probiotic indicate similar effects.

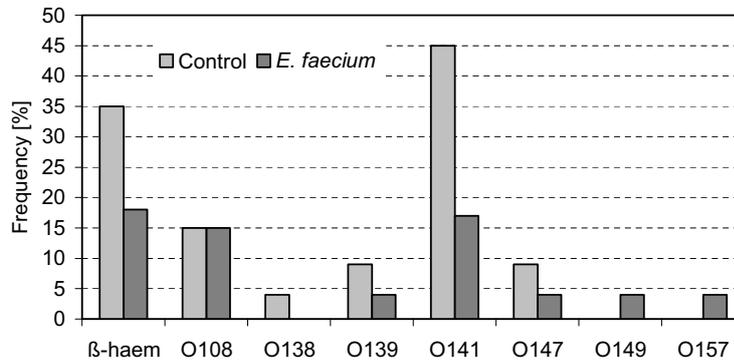


Figure 3: Isolation frequency of β -haemolytic and selected *E. coli* serogroups in control piglets and piglets receiving *Enterococcus faecium* NCIMB 10415

Determination of total anaerobic bacteria and coliform bacteria indicated no significant impact of probiotic treatment (*E. faecium*) on these bacterial populations in intestinal content of piglets. However, a more detailed characterization of the *E. coli* isolates by the frequency (isolation rate) of β -haemolytic *E. coli* and of various serogroups of *E. coli* revealed important differences between the treatment groups (Scharek et al., 2005; Figure 3).

The isolation frequency of total β -haemolytic *E. coli* was reduced up to 50 % in piglets of the probiotic group compared to untreated animals. In addition, the isolation frequency of the O141 serogroup of *E. coli* was also reduced at the same extent in probiotic-treated piglets. This observation is of particular interest since the O141 serovar group of *E. coli* is one of four classical serogroups associated with post-weaning diarrhoea and edema disease in piglets (Bertschinger and Fairbrother, 1999; Frydendahl, 2002), and therefore might explain the observed reduction of incidence or diarrhoea in the probiotic treated piglets.

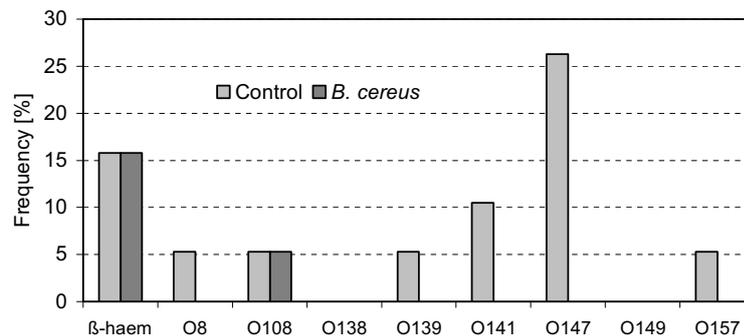


Figure 4: Isolation frequency of β -haemolytic and selected *E. coli* serogroups in control piglets and piglets receiving *Bacillus cereus* var *toyoi*

In piglets receiving diets supplemented with *B. cereus* var. *toyoi*, no isolates of the O141 serogroup were detected, while the isolation frequency of this serogroup was at least 10 % in control animals (Figure 4). Even more pronounced was the reduction of isolation frequency of the O147 serogroup. This latter observation is also noteworthy since the O147 serogroup is among the most commonly isolated enterotoxigenic *E. coli* (ETEC) strains of porcine origin associated with diarrhoea in neonatal pigs (Sojka, 1971; Blanco et al., 1991; Garabal et al., 1996).

Furthermore, it was shown for the first time that using *E. faecium* NCIMB 10415 as a feed additive for sows and piglets, the carry-over infection of piglets by chlamydia from chlamydia-positive sows was reduced. While 85 % of piglets from the control group were found to be chlamydia-positive, chlamydia were found in only 60 % of piglets from the probiotic-treated group (Pollmann et al., 2005).

Altogether, these findings indicate a reduced occurrence of pathogenic bacteria in the digestive tract of piglets treated with these probiotic micro-organisms.

Effects on structure and function of the intestinal mucosa

Because some studies have shown that modifications of the gut morphology (Görke, 2000) and transport kinetics of nutrients in the jejunum (Breves et al., 2000) might be involved in the overall effects of probiotics we included systematic measurements of such parameters in our experiment. However, no modifications due to the probiotic treatment could be detected. Neither villi length nor crypt depth appeared to be affected by the probiotic treatment in the various sections of the intestine at the different ages of the piglets. Likewise, other parameters also showed no specific modifications.

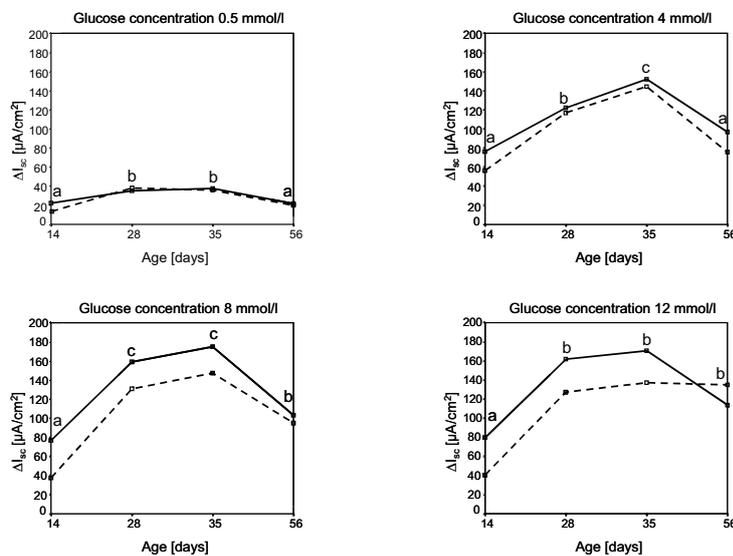


Figure 5: Short circuit current (I_{sc}) response in jejunum of piglets to luminal increasing concentrations of glucose. Comparison of tissues from control and probiotic treated animals (*E. faecium* NCIMB 10415)

On the other hand, an increased stimulation of sodium-dependent glucose transport rates with increasing glucose concentration was observed in jejunal epithelium (*in vitro* Ussing chamber technique) for piglets receiving *E. faecium* NCIMB 10415 (Figure 5). These results were consistent with earlier findings of Breves et al. (2000) using *B. cereus* var. *toyoi* or *Saccharomyces boulardii* as feed additive in weaned piglets. However, a discrepancy exists in our study in so far as we did not observe a similar effect with *B. cereus* var. *toyoi*. An improved glucose stimulation rate might be explained by an increased absorptive surface; however, this was not supported by morphometric measurements in the same animals. It remains possible that the observed stimulation of glucose transport is mediated at a regulatory or the expression level of the sodium-glucose co-transporter SGLT1, which will be the subject of future studies.

Effects on immunological parameters

Until now it has remained unclear whether the observed health promoting effect of probiotics is a result of a stimulation of the immune system or of other properties of the applied micro-organisms. In particular, it has not been investigated whether the immune system of pigs can be affected at all by probiotic treatments. A set of data on the effect of both probiotics was generated and most of the results from the study with the *E. faecium* NCIMB 10415 probiotic have recently been published (Scharek et al., 2005). Because of the large amount of data, these results will only be summarized here.

Neither of the two probiotics affected the population of lymphocytes in Peyers patches. The population of intraepithelial lymphocytes, however, showed significant, systematic changes at several sampling times in both probiotic studies. Interestingly, *E. faecium* NCIMB 10415 and *B. cereus* var. *toyoi* were found to act on the immune system in different ways. An other important observation was that effects on the immune response were most pronounced at an early stage of the development, i.e. at weaning (*B. cereus* var. *toyoi*) or even at day 14, before probiotic diet was ingested by the piglets (*E. faecium* NCIMB 10415, Figure 6).

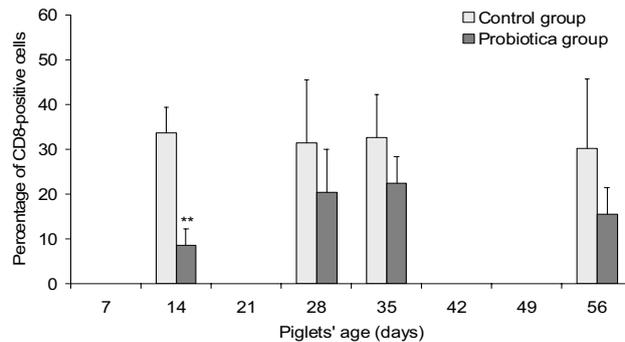


Figure 6: CD8-positive T-cells in the jejunum epithelium of control piglets and piglets from the *E. faecium* NCIMB 10415 treatment group. The probiotic was included in all diets of gestating and lactating sows as well as in the prestarter and starter diets of piglet

The main results may be summarized as following:

Effects of *E. faecium* NCIMB 10415

- Intraepithelial CD8-positive cells (cytotoxic T-cells) and IgG concentration in blood: REDUCED
- The effects are most pronounced before weaning
- No effects on lymphocyte populations in Peyers patches
- These are most likely secondary effects due to a reduced microbial challenge (see above: 2.3)

Effects of *Bacillus cereus* var. *toyoi*

- Intraepithelial populations of CD45-positive cells (leucocytes), CD3 CD8-double positive cells (cytotoxic T cells) and TcR1-positive cells ($\gamma\delta$ T-cells): INCREASED
- No effects on lymphocyte population in Peyers patches
- The effects of *B. cereus* probiotic suggest a direct immune stimulation

Summary

Two probiotic micro-organisms authorized in the EU as feed additives (*Enterococcus faecium* NCIMB 10415 and *Bacillus cereus* var. *toyo*) were applied to gestating and lactating sows as well as to suckling and weaned piglets. Both probiotics were transferred very early *a.p.* from the sow to the piglets and colonized the whole intestinal tract of the piglets. While effects on animal performance were marginal and not consistent, incidence of post-weaning diarrhoea was reduced by both probiotics significantly. The mode of action of the probiotics includes modified and less diverse intestinal microbiota, reduced exposure to pathogenic bacteria, modified nutrient transport kinetics by jejunal epithelia and modifications of the immune system. The two utilized probiotics affect the immune system in a different way.

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Neuroprotective effects of dietary vitamin E and Ginkgo biloba

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Summary

A wide spectrum of beneficial activity to human health has been advocated for antioxidant nutrients. More recently the ability of antioxidants to affect gene expression and cell response has been reported, providing a novel mechanistic perspective on their biological activity. An imbalance in the oxidant antioxidant homeostasis may be detrimental to the organism and contribute to the pathogenesis of neurodegeneration. The efficacy of different antioxidants such as vitamin E and Ginkgo biloba to influence positively the molecular mechanisms implicated in age-related diseases such as Morbus Alzheimer is becoming increasingly important. Differential changes in the expression of several groups of genes might be a key point underlying the complex behaviour of antioxidants. Our studies reveal that both vitamin E and Ginkgo biloba have notable neuromodulatory activity and illustrate the utility of genome-wide expression monitoring to investigate the biological actions of antioxidants in vivo. This approach may lead to better insights into the role of dietary antioxidants in healthy aging, thereby offering a novel strategy in nutrition research.

Vitamin E and neuroprotection

Vitamin E (VE) is considered the most important, natural occurring, hydrophobic antioxidant (Packer *et al.*, 2001; Rimbach *et al.*, 2002), and its role in the maintenance of brain function is becoming increasingly recognised. A number of cell culture and animal studies using models of neurodegeneration have suggested a protective role for vitamin E in the reduction of oxidative damage, glycation and amyloid beta toxicity (Usuki *et al.*, 2001). The symptoms of VE deficiency in alpha-tocopherol transfer protein (α -TTP) knock out mice include neurological impairment, ataxia and dysfunctional reflexes accompanied by increased oxidative damage in the brain tissue. Vitamin E supplementation have been shown to decrease oxidative damage and prevents neurodegeneration (Yokota *et al.*, 2001).

Evidence for a role of vitamin E in protecting against the onset of AD and its progression in humans is based largely on epidemiological data, where lower plasma, brain and cerebrospinal fluid (CSF) vitamin E levels were detected in patients relative to a matched control group (Grundman *et al.*, 2002). In addition Morris and co-workers observed a lower rate of development of AD in individuals who regularly consumed vitamin E supplements relative to the non-supplement users in a 4 year prospective study of non-AD elderly participants at baseline (Morris *et al.*, 1998).

Until yet only a very limited number of clinical trials has examined the effects of vitamin E supplementation on disease progression in AD patients as summarized in Table 1.

Table 1: Effects of vitamin E supplementation on disease progression in AD patients

	VE application	Duration	Design	Results
Sano et al. 1997	2000 IU/day	3 months	341 patients placebo-controlled, double-blind, randomized, multicenter	decreased progression of AD
Onofrij et al. 2002	2000 IU/day	6 months	60 patients controlled clinical trial, randomized	delayed onset of AD

In the Alzheimer's Disease Cooperative Study, individuals with moderate AD were randomly assigned to consume either 2000 IU/d VE or a control treatment (Sano *et al.*, 1997). A significant delay in the onset of dementia and institutionalisation was evident in the vitamin E supplemented group. Although there is an ever increasing body of evidence to suggest that vitamin E may prevent or delay the onset of AD, the molecular mechanisms remain poorly understood. It is probable that part of the benefit is attributable to a role of vitamin E as an antioxidant, thereby reducing oxidative modification within the neuronal cell. However it is likely that VE also mediates its biological effect through non-antioxidant molecular targets in the brain tissue. The first observations of a cell signalling role for VE were made in 1991 by Azzi and co-workers (Boscoboinik *et al.*, 1991) who demonstrated that VE inhibited protein kinase C activity and cell proliferation in cultured cells.

Advances in microarray technology have allowed to investigate genes differentially expressed in various tissues including liver (Barella *et al.*, 2004, Rimbach *et al.*,

2004), testes (Rota *et al.*, 2004), prostate (Siler *et al.*, 2004), cortex (Gohil *et al.*, 2003) and total brain homogenates (Roy *et al.*, 2002) in response to VE deficiency thereby offering the possibility of more insight into the molecular functions of VE. However, the impact of VE on gene expression and cell signalling in the hippocampus has yet not been fully defined.

Therefore we examined the impact of medium term (9 months) VE deficiency on hippocampal gene expression using gene chip technology, with the response assessed in each animal using an individual DNA microarray as summarized in Fig. 1.

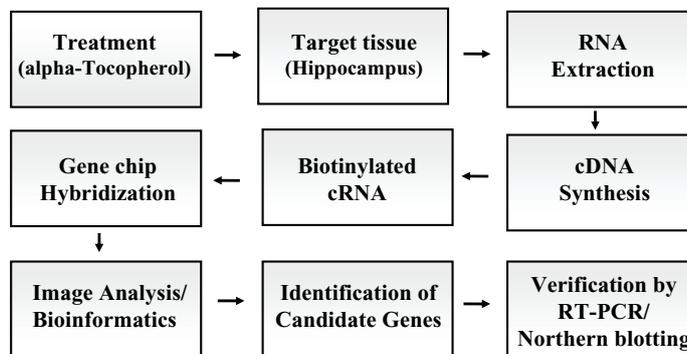


Figure 1: Schematic representation of the analytical steps involved in a gene chip experiment

Male albino rats were fed either a vitamin E deficient (VE⁻) or standard diet (VE⁺) (60 mg/kg feed) for a period of 9 months. Rats were sacrificed, the hippocampus removed and genes expression established in individual animals. Our choice of tissue was guided by the fact that the hippocampus is known to play an essential role in cognition and in particular in memory processing being a centre for the short-term memory before the information is transferred to the cerebral cortex for permanent storage. Furthermore it is considered the principal side of dysfunction in AD.

Several VE sensitive genes, which may be important in the pathophysiology of neurodegenerative diseases, were identified supporting a protective role for VE in the prevention/delay of age-related neurological conditions. VE deficiency showed to have a strong impact on genes expression in the hippocampus. An important number of genes found to be regulated by VE were associated with hormones and hormone

metabolism, nerve growth factor, apoptosis, dopaminergic neurotransmission, and clearance of amyloid beta (Abeta) and advanced glycated endproducts (AGE). In particular vitamin E strongly affected the expression of an array of genes encoding for proteins directly or indirectly involved in the clearance of amyloid beta, changes which are consistent with a protective effect of VE on AD progression.

Vitamin E sensitive genes

Amyloid beta peptide (Abeta) is a 40-42 amino acid peptide, generated by the proteolytic cleavage of its precursor, amyloid beta precursor protein (APP). Cleavage of APP can occur through two different pathways, catalysed by three different classes of secretases. Alpha secretases produce amyloid beta in the soluble form (sAbeta), a form which is normally secreted in extracellular fluids and represents a normal component of plasma and CSF. When the hydrolysis of APP is catalysed by beta or gamma secretases, the insoluble Abeta protein is produced. Abeta is the major component of senile plaques, the extracellular, cerebrovascular fibrous deposits, representing the major hallmark of neurodegeneration in Alzheimer's disease (Nunan *et al.*, 2002).

VE deficient rats showed a decreased expression of amyloid beta precursor protein binding protein member 1 (Table 2). This protein binds the APP stabilizing it and slowing down its cellular processing. In this way the formation of Abeta fragments is inhibited (Sastre *et al.*, 1998; Borg *et al.*, 1998).

In the current study, dietary VE⁻ resulted in lower levels of the mRNA encoding for prealbumin, a precursor of albumin. Albumin is a major transport protein, and is the most abundant protein in plasma and CSF. Abeta is transported, in the plasma, mainly bound to albumin. As discussed above, it has been reported that higher albumin (and transhyretin) levels in the brain, resulting from IGF-I administration, correlated with decreased Abeta brain levels (Biere *et al.*, 1996).

VE deficient rats also exhibited lower mRNA levels of megalin (glycoprotein 330). Megalin is an endocytic-membrane glycoprotein, which belongs to the LDL receptor (LDLR) family and is expressed on the apical surface of epithelial cells of several organs including kidney, lung and brain. The binding of ligands to megalin is followed by endocytosis. With this mechanism ligands are targeted to lysosomes for degradation, or are transported across the cell (Christensen *et al.*, 2002). It is

involved in the endocytic uptake of lipoproteins, carrier proteins, iron, calcium, enzymes and drugs. Megalin is also involved in the regulation of thyroid hormone production since it mediates the uptake and transcytosis of thyroglobulin (Christensen *et al.*, 2002). Its deficiency in mice is characterized by abnormalities in epithelial tissues and defective forebrain development (Willnow *et al.*, 1996).

ApoJ (also called clusterin) binds Abeta and promotes amyloid beta plaque formation (DeMattos *et al.*, 2002). Megalin, by binding the Abeta-apoJ complexes, leads to their lysosomal degradation and contributes to Abeta clearance (Hammad *et al.*, 1997). During the aging process, long-lived proteins are characterised by specific post-translational modifications, e.g. advanced glycation end product formation (AGE). AGEs have been detected in a wide variety of diseases including PD and AD (Reddy *et al.*, 2002; Sasaki *et al.*, 1998). Since it has been shown that megalin has the capability of mediating the endocytosis and degradation of AGEs *in vitro* (Saito *et al.*, 2003), it is possible that decreased megalin expression would contribute to an increase in AGEs concentration in neuronal cells *in vivo*.

Table 2: Genes differentially regulated by Vitamin E and Ginkgo biloba in the hippocampus (H) and cortex (C) of rats and mice

Vitamin E sensitive genes (rats)	Ginkgo biloba sensitive genes (mice)
Amyloid beta precursor protein binding protein member 1 (H)	Neuronal tyrosine/threonine phosphatase 1 (H)
Preproalbumin (H)	AMPA-2 (H)
Megalin (H)	Prolactin, growth hormone (H)
Clusterin (H)	Transthyretin (C)

Gene regulatory activity of Ginkgo biloba

Ginkgo biloba extract EGb 761 is commonly used to combat a variety of neurological disturbances such as Alzheimer's disease or various common geriatric complaints including vertigo, depression, short term memory loss, hearing loss, lack of attention, or vigilance (Le Bars *et al.*, 1997; Rimbach *et al.*, 2003). Various human intervention studies regarding the effect of Ginkgo biloba on cognitive function are summarized in Table 3.

To gain further insights into the biochemical effects of Ginkgo biloba, we profiled the transcriptional effects of the extract on the brains of mice using oligonucleotide

microarrays (Watanabe *et al.*, 2001). These microarrays represent all sequences (~6000) in the Mouse UniGene database that have been functionally characterized, as well as ~6000 expressed sequence tags clusters. The effects of Ginkgo biloba extract EGb 761 on gene transcription were measured in the hippocampus and cerebral cortex of adult female mice (n=10, per group) who were fed a diet either with or without Ginkgo biloba (300 mg EGb 761 per kg diet). After the 4-week diet regimen with EGb 761 the hippocampi and cortices were removed and pooled with respect to both tissue type and treatment. Of the 12,000 combined genes and ESTs represented on the array, only 10 changed in expression level by three fold or more and all were upregulated. These findings are summarized in Table 2. In the cortex, mRNAs for neuronal tyrosine/threonine phosphatase 1, and microtubule associated tau were significantly enhanced. Both proteins are associated with the formation/breakdown of intracellular neurofibrillary tangles, a hallmark lesion of Alzheimer's disease. Hyperphosphorylated tau has been found to be the major protein of these neurofibrillary tangles, possibly because of an imbalance of tau kinase and phosphatase activities in the affected neurons (Iqbal *et al.*, 1998).

Table 3: Effects of oral supplementation of EGb 761 on dementia in AD patients

	EGb 761 application	Duration	Design	Results
Kanowski et al. 1996	240 mg/day	24 weeks	156 patients placebo-controlled, double-blind, randomized, multicenter	clinical efficacy in the treatment of patients with dementia
Le Bars et al. 1997	120 mg/day	52 weeks	244 patients placebo-controlled, double-blind, randomized, parallel-group, multicenter	stabilization and partial improvement of cognitive and social functioning up to 6-12 months
Maurer et al. 1997	240 mg/day	3 months	20 patients placebo-controlled, double-blind, randomized, parallel-group	improvement of attention and memory
Kanowski and Hoerr 2003	240 mg/day	24 weeks	216 patients placebo-controlled, double-blind, randomized, parallel-group, multicenter	improvement of cognitive function

Hyperphosphorylated tau isolated from brains of those with Alzheimer's disease has been shown to be efficiently dephosphorylated in vitro by protein phosphatases 1, 2A and 2B. Additionally, selective inhibition of protein phosphatase 2A by okadaic acid

in metabolically competent rat brain slices has been shown to induce a hyperphosphorylation and accumulation of tau like that in Alzheimer's disease (Gong *et al.*, 2000). Thus, upregulation of neuronal phosphatase 1 by EGb 761 could play a neuroprotective role in the brain.

The expression of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid-2 (AMPA-2), calcium and chloride channels, prolactin, and growth hormone (GH), all of which are associated with brain function were also upregulated. Within the past decade, studies have revealed that GH may exert significant effects on the central nervous system. Cognitive impairments are well known hallmark features of GH deficiency, and clinical studies have reported psychological improvements (in mood and well being) and beneficial effects on certain functions including memory, mental alertness, motivation, and working capacity in adults receiving GH replacement therapy. Moreover, GH therapy in children deficient in this protein have been reported to produce marked improvement in their behaviour (Nyberg *et al.*, 2000).

In the hippocampus only transthyretin mRNA was induced. Transthyretin plays a role in the transport of thyroxine and retinol binding protein in the brain. Thyroid hormones regulate neuronal proliferation and differentiation in discrete regions of the brain during development and are necessary for normal cytoskeletal outgrowth (Porterfield *et al.*, 2000). Transthyretin has also been shown in vitro to sequester amyloid beta protein and prevent amyloid beta aggregation from arising in amyloid formation (Tsuzuki *et al.*, 2000). In addition, transthyretin levels in cerebrospinal fluid have been found to be significantly decreased in Alzheimer's disease patients. Thus, one mechanism whereby EGb 761 may exert neurological effects is the modulation of transthyretin levels, and as a consequence, by either hormone transport or amyloid beta sequestration in the brain.

Ginkgo biloba and synaptic transmission and plasticity

In addition to our gene array work we have recently studied the acute and chronic effect of EGb 761 on synaptic transmission and plasticity in hippocampal slices from young adults (8-12 weeks) and aged (18-24 months). C57Bl/6 mice were tested because hippocampal plasticity is believed to be a key component of memory. Acutely applied EGb 761 significantly increased neuronal excitability in slices from aged mice by reducing the population spike threshold and increased the early phase

of long-term potentiation, though there was no effect in slices from young adults (Williams *et al.*, 2004). In chronically treated mice fed for 30 days with an EGb 761-supplemented diet, EGb 761 significantly increased the population spike threshold and long-term potentiation in slices from aged animals, but had no effect on slices from young adults. The rapid effects of EGb 761 on plasticity indicate a direct interaction with the glutamatergic system and raise interesting implications with respect to a mechanism explaining its effect on cognitive enhancement in human subjects experiencing dementia.

Conclusion

In conclusion, aging population demographics has resulted in an ever increasing incidence of age-related neurodegenerative disorders. However, research into dietary strategies which may delay the progression of the condition are distinctly lacking, relative to other chronic conditions such as coronary heart disease and cancer. A limited number of recent clinical studies have associated supplementation with vitamin E and Ginkgo biloba with the amelioration of some neurodegenerative disease symptoms, which support earlier epidemiological evidence and cell culture and animal experiments. The mechanisms underlying the possible protective effects of vitamin E and Ginkgo biloba remained largely unclear. The current review demonstrates that both vitamin E and Ginkgo biloba affect the expression of an array of genes encoding for proteins directly or indirectly involved in the prevention of Alzheimer's disease. Also potential synergistic interactions between Vitamin E and ginkgo biloba in the prevention of Morbus Alzheimer should be taken into account in future investigations. The current study adds validity to the potential for dietary antioxidants protecting against neurodegeneration and in part provides an understanding of the underlying molecular mechanisms.

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Encapsulation of vitamins and ingredients: Demands and technological options

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During the last years growing markets for human convenience food as well as the nutritional needs in modern animal production let to an increasing demand for products based on encapsulated vitamins and other ingredients.

Beneficial effects such as a higher shelf-life due to protection against environmental effects, e.g. air, light, and especially a controlled release of the substances inside the intestinal tract of both humans and animals can be achieved by encapsulation.

From the technological point of view two major challenges has to be faced for the manufacture of suitable encapsulated products. First, depending on the kind of protection needed and the conditions under which the encapsulated substances should be released in a controlled way, e.g. temperature, acidic or neutral pH, a suitable encapsulation matrix has to be chosen under the limited number of substances allowed for nutritional uses. Second, the encapsulation technology should be able to manufacture the product in the desired concentration inside the chosen matrix, the desired form, size distribution and stability as well as for low costs.

Different encapsulation strategies mainly based on controlled release demands and technologies for product manufacture will be presented and their pros and cons will be discussed.

Influence of juices rich in carotenoids on biomarkers of inflammatory status and oxidative stress.

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Introduction

A high consumption of fruits and vegetables has been correlated with reduced risks for certain diseases, such as cancer, coronary heart disease, stroke and dementia. The intake of plant foods has been associated with an increase of plasma levels of vitamins, carotenoids and polyphenolic compounds. Because oxidative stress plays an important role in most disease processes and aging, the potential health benefits of fruits and vegetables are attributed to their potential antioxidant capacity.

Chang et al. (2005) showed that patients with acute ischemic stroke had lower levels of carotenoids including lycopene and higher levels of inflammation markers than a healthy control group. A intake of 5-10 mg lycopene per day was recommended by Rao and Shen (2002) due to their findings of significant reduction of lipid and protein oxidation by low dose intake of lycopene. Recently, the effect of the consumption of orange juice and gaspacho, products with a high amount of vitamin C, on oxidative stress and inflammatory biomarkers was studied (Sánchez-Moreno et al., 2003, 2004). The authors reported that an intake of 500 mL of these products for two weeks reduced the levels of biomarkers studied. They discussed the beneficial effect exclusively to the amount of vitamin C consumed. Thus, the aim of the presented study was to investigate if other components, such as carotenoids, present in fruits and vegetables, also participate in the reduction of oxidative stress and inflammatory processes.

Exposure of proteins to the attacks of oxygen radicals results in multiple changes in the target molecule, resulting in a loss of structural or enzymatic activity of the pro-

tein. The common method to assess the oxidative damage of proteins is to measure the concentration of carbonyl groups (Levine et al., 1990). To quantify lipid peroxidation, peroxidation products mainly from polyunsaturated fatty acids (PUFA) are measured. Initial products of damage are hydroxylated conjugated dienes. These decompose either into various aldehydes (measured as malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS)), in case of arachidonic acid into isoprostanes. Previous studies have shown that diets with low levels of antioxidants are associated with elevated levels of 8-isoprostanes (Morrow and Roberts, 1997).

Biomarkers of inflammation are also associated with several chronic diseases. Interleukin-1 β is critical for mediating host responses of infection and injuries. Disease states with enhanced levels of IL-1 β are arthritis, arteriosclerosis, allergic diseases and some types of cancer (Rosenwasser et al., 1998)

Materials and Methods

Study design

Twenty-four healthy volunteers (20 women, 4 men) with normal body weight (BMI = 21.5 ± 2.9 kg/m², age = 23.2 ± 2.4 years) participated in the 4-weeks-trial. The participants were neither pregnant, lactating or had any chronic illness nor were taking any vitamin or mineral supplement. Subjects continued their habitual diets during the study, with some precise instructions. They were asked to exclude foods rich in lycopene and foods rich in vitamin C (citrus and tropic fruits and strawberries) throughout the trial and to consume three portions of fruits or vegetables each day. After a two weeks depletion period, the participants were divided in two groups. The investigation group obtained 250 mL tomato juice without vitamin C fortification twice daily (41.8 mg lycopene/L and 90 mg vitamin C/L). The control group obtained the same amount of juice but with vitamin C fortification (40.72 mg lycopene/L, 870 mg vitamin C/L). Both juices were prepared at Juver, S.A., Spain. Fasting blood and urine samples were taken prior to the depletion period and after 2 weeks each (T-2, T0, T+2) and stored at -80°C until analysis, in case of plasma after prior separation of red blood cells by centrifugation. Urine samples were collected for 24 hours starting the day prior to blood withdrawal.

Plasma analysis

Vitamin C: Vitamin C was determined photometrically according to the method of Speitling et al. (1992).

Carotenoids: Carotenoids were extracted by hexane using echinenone as internal standard and analysed using a HPLC-method according to Böhm (2001) with slight modifications.

Antioxidant activity: For the **TEAC** (trolox equivalent antioxidant capacity) assay ABTS radical solution was prepared using potassium persulfate as radical generator. The radical solution was adjusted to an absorbance of $0,7 \pm 0,1$ at $\lambda = 734$ nm with phosphate buffer and samples were measured 1 min after adding to the radical solution. **FRAP** (ferric reducing activity of plasma) was determined according to Schlesier et al. (2002).

Carbonylated proteins: The content of carbonylated proteins was measured as described by Levine et al. (1990) using 2,4-dinitrophenylhydrazine. Total protein content as reference was measured in the same sample as described by Reznick and Packer (1994) with bovine serum albumine (BSA) as standard compound.

TBARS: Thiobarbituric acid (TBA, 0,67%) and trichloroacetic acid (20%) were added to plasma samples. Then the samples were incubated for 20 min at 99 °C developing a red colour which was measured at $\lambda = 532$ nm. Results were determined using the specific molecular coefficient of the TBA-malondialdehyde-complex of $1,56 \cdot 10^5 \text{M}^{-1}$.

Interleukin-1 β : IL-1 β was determined by a commercial kit (Cayman, cat. no: 583311). Values after 6 h of incubation were considered as results.

Juice analysis

Vitamin C was determined as described above.

Carotenoids: Carotenoids were extracted from the product by MeOH/*tert*-butyl-methyl-ether (TBME, 1+1v/v, +1%BHT) using 8-*apo*'-carotenal as internal standard. HPLC conditions were the same as described above.

Antioxidant activity: TEAC and FRAP were measured as described above.

Statistical analysis

All values are presented as means \pm SD. Analysis was performed using SPSS 13.0 for Windows. Differences between variables were tested for significance by using the general linear model (GLM) for the 2-way ANOVA procedure, using a level of signifi-

cance of $p < 0.05$. Differences between both groups were tested by using the 1-way ANOVA ($p < 0.05$). Correlations were determined by using Spearman's correlation.

Results and Discussion

Nutritional values and concentrations of vitamin C and lycopene as well as antioxidant capacity of tomato juices are presented in **Table 1**.

Table 1. Composition of tomato juice (*values are means \pm SD*)

	Units/100 mL	
	Intervention	Control
Energy, kJ ¹⁾	82	82
Proteins, g ¹⁾	0.8	0.8
Carbohydrates, g ¹⁾	4.0	4.0
Fat, g ¹⁾	0.0	0.0
Vitamin C, μmol	9.1 ± 0.3	86.9 ± 3.3
Total lycopene, μmol	41.8 ± 0.9	40.7 ± 1.9
TEAC, mmol TE		
FRAP, μmol	234 ± 2	1483 ± 48

¹⁾ values from package

Vitamin C in plasma showed no difference between the groups ($p > 0.05$) at T-2 and T0. The concentration significantly decreased in both groups during the depletion period and significantly increased by 58.5% after consumption of tomato juice in the control group, but not in the intervention group (+ 2.5%). At T+2, vitamin C content in plasma was significantly higher in the control group than in the intervention group ($p < 0.01$). Results are shown in **Figure 1** and **Table 2**.

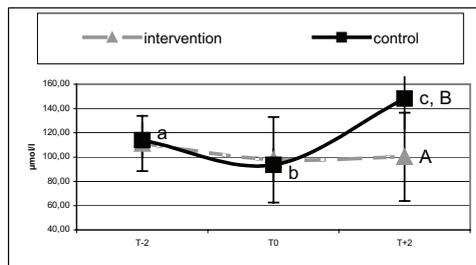


Figure 1. Plasma vitamin C concentrations in men and women at beginning (T-2), end of depletion (T0) and end of intervention (T+2), points with different letters are significantly different ($p < 0.05$).

Plasma lycopene levels were not different between both groups at T-2, but at T0 and at T+2. Both groups demonstrated a significant decrease ($p < 0.019$) during the

depletion period and a significant increase ($p < 0.01$) in the intervention group due to lycopene absorption from the juice. In both groups, the lycopene concentration was higher ($p < 0.01$) at T+2 than at the beginning (T-2) (**Table 2**). Increase of lycopene concentration during the intervention phase indicates a good bioavailability of lycopene from tomato juice.

Table 2. Plasma vitamin C, total lycopene, antioxidant capacity (TEAC, FRAP), carbonylated proteins, TBARS and IL-1 β at T-2, T 0 and T+2 (*values are means \pm SD, n=12/group*)

		intervention			control		
		T-2	T 0	T+2	T-2	T 0	T2
Vitamin C	$\mu\text{mol/L}$	111 \pm 22	98 \pm 34	100 \pm 35	114 \pm 14	96 \pm 13	148 \pm 25
Total lycopene	$\mu\text{mol/L}$	0.73 \pm 0.27	0.47 \pm 0.19	1.04 \pm 0.41	0.70 \pm 0.26	0.33 \pm 0.12	0.83 \pm 0.22
TEAC	mmol TE/L	3.6 \pm 1.5	3.6 \pm 1.6	3.6 \pm 1.4	3.4 \pm 0.4	3.3 \pm 0.4	3.4 \pm 0.3
FRAP	mmol/L	0.84 \pm 0.18	0.82 \pm 0.16	0.85 \pm 0.22	0.80 \pm 0.28	0.83 \pm 0.24	0.82 \pm 0.25
Carbonylated protein	nmol/mg P.	0.63 \pm 0.11	0.63 \pm 0.12	0.57 \pm 0.06	0.59 \pm 0.11	0.68 \pm 0.11	0.66 \pm 0.13
TBARS	$\mu\text{mol MDA/L}$	0.51 \pm 0.09	0.54 \pm 0.11	0.54 \pm 0.10	0.55 \pm 0.09	0.56 \pm 0.13	0.56 \pm 0.18
Interleukin-1 β	ng/L	4.2 \pm 2.3	3.9 \pm 2.4	4.4 \pm 1.8	9.7 \pm 9.2	13.2 \pm 9.2	8.3 \pm 6.7

Juice consumption did not improve antioxidant capacity in plasma (FRAP values), but showed a significant increase after intervention in the control group (TEAC values). Values did not differ between the groups. This is consistent with other studies. Böhm and Bitsch (1999) reported no change in TEAC values of plasma after ingestion of tomatoes, tomato juice and oleoresin capsules. Lee et al. (2000) reported that only addition of olive oil but not sunflower oil to an tomato product raised antioxidant activity of plasma. But lycopene levels increased similar in both groups. These effects could be due to the tests used. Lycopene is a lipophilic compound, so its impact in a hydrophilic test system is not clear. A significantly higher TEAC value at the end of the study in the control group demonstrates an impact of vitamin C on antioxidant capacity of plasma, although there was no statistically significant difference between values of both groups.

Markers of oxidative stress showed contradictory results. In case of carbonylated proteins in plasma there was a significant decrease in the intervention group between the beginning and the end of the study (from 0.63 \pm 0.09 nmol/mg protein at T-2 to 0.60 \pm 0.08 at T+2) versus an increase in the control group (from 0.59 \pm 0.11 at T-2 to 0.66 \pm 0.13 at T+2). This effect was not caused by the intervention phase. TBARS demonstrated no change in the control group but a significant increase in the intervention group due to a change in the depletion phase. Concentration of interleukin-1 β

in plasma was different between the groups at all three measurement points. There were no changes throughout the study in the intervention group but an significant decrease in the control group due to juice consumption.

Sánchez-Moreno et al. (2003) constituted a protective effect of vitamin C on oxidative stress and inflammation due to an inverse correlation between vitamin C in plasma and levels of isoprostane (8-epiPGF_{2α}) and a decrease in prostaglandine levels in plasma after two weeks of gaspacho consumption (500 mL/day). However, investigating other biomarkers of oxidative stress and inflammatory processes the here presented results cannot come to the same conclusion yet, neither for vitamin C impact nor for lycopene. Further studies will be carried out investigating other parameters of oxidative stress and inflammation.

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Enrichment of cows' milk with natural or synthetic vitamin E

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Introduction

The effect of natural and supplemental vitamins in feed on the vitamin content in cows milk has been addressed in many studies. α -Tocopherol has attracted appreciable interest regarding prevention of oxidation problems in milk fat (Nicholson & St-Laurent, 1991; Charmley & Nicholson, 1994). α -Tocopherol has also been related to health problems such as mastitis, and to immune defence (Politis *et al.*, 1995; Hogan *et al.*, 1996).

The mechanism involved in the translocation of α -tocopherol from feed to milk is poorly understood. Absorption in the small intestine is believed to be passive diffusion and highly facilitated by dietary fat intake (Charmley & Nicholson, 1994). After incorporation into chylomicrons tocopherols are transported in the blood plasma by low density as well as high density lipoproteins (Senaidy, 1996). The process involved in the final transportation from plasma lipoproteins into milk fat is not known, but only a few percent is secreted into milk (Hidiroglou, 1989; Weiss & Wyatt, 2003). Catignani & Bieri (1977) found a α -tocopherol binding protein in rat livers. Burton & Traber (1990) argued that a α -tocopherol binding protein controls the preferential biodiscrimination of 2R- α -tocopherol over 2S- α -tocopherol in incorporation into chylomicrons in the liver, resulting in a natural to synthetic α -tocopherol ratio of 2:1 in rats and humans. Recently Meglia *et al.* (2006) showed that RRR- α -tocopherol is the predominant stereoisomer in plasma and milk from cows. The purpose of the present investigation was to elucidate different possibilities for enrichment of cows' milk with vitamin E, with special focus on the natural and synthetic forms of α -tocopherol.

Materials and Methods

Three experiments was conducted with the experimental cow herd at the Danish Institute of Agricultural Sciences.

In experiment 1 twelve Holstein cows in midlactation was used. All cows were fed a TMR diet consisting of clover grass silage (28%), corn silage (18%), sugar beet pellets (10%), barley straw (3%) and concentrates (41%). During a three week period the cows in addition were fed either 0.3 L rapeseed oil or 0.3 L of a mixture consisting of rapeseed lecithin, rapeseed fatty acids and natural vitamin E (1650 mg RRR- α -tocopherol/kg) so this group was supplemented with additional 500 mg RRR- α -tocopherol. Blood and milk samples were taken at the beginning of the experiment and again at the end of the experiment.

In experiment 2 forty cows was enrolled in a two times two factorial experiment. The first factor was supplement of 300 mg *all-rac*- α -tocopheryl acetate or 300 mg *all-rac*- α -tocopheryl acetate plus 1450 mg RRR- α -tocopherol pr cow per day. The second factor was 1.2 kg rapeseed oil or sunflower oil from rapeseed or sunflower cake. This experiment lasted 5 weeks and blood and milk samples were taken at the beginning of the experiment, after 1 week and again after 5 weeks.

In experiment 3 four cows was injected intramuscularly (i.m.) with 2.5 g of *all-rac*- α -tocopheryl acetate (IDO-E VET) and subsequently blood and milk samples were taken for 11 days. In addition six cows were daily fed 3000 mg *all-rac*- α -tocopheryl acetate for 16 days, after which blood samples were taken and analysed for stereochemical composition of α -tocopherol and these results were compared with data from rats.

Sampling procedure and tocopherol analyses were performed as previous described (Jensen et al., 1999) and analyses of stereoisomers of methyl ethers of α -tocopherol were performed as described by Lauridsen & Jensen (2005).

Results and Discussion

Experiment 1.

Supplementation of the vitamin E rich oil (Leci E) as top dressing to lactating cows increased α -tocopherol content in both plasma and milk. Addition of rapessed oil alone increased α -tocopherol only in plasma ($P < 0.01$), but not in milk (Table 1). Supplementation with Leci E on the other hand increased α -tocopherol content in both plasma and milk and the α -tocopherol content in milk was significantly higher in the Leci E supplemented group compared to the rapeseed oil group expressed both as concentration in milk and milk fat (Table 1).

Table 1. Plasma and milk concentration of α -tocopherol after supplementation of cows with 500 mg RRR- α -tocopherol together with 0.3 L rapeseed lecithin and fatty acids compared with 0.3 L pure rapeseed oil for 21 days (n = 6; means \pm SEM)

α -Tocopherol	Initial		21 Days		
	Plasma	Milk	Plasma	Milk	Milk fat
	$\mu\text{g/ml}$	$\mu\text{g/g}$	$\mu\text{g/ml}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Rapeseed oil	4.1 \pm 0.4	0.70 \pm 0.12	6.8 \pm 0.6	0.66 \pm 0.04	15.4 \pm 0.7
Leci E	4.0 \pm 0.3	0.70 \pm 0.08	7.6 \pm 0.7	0.86 \pm 0.08	22.0 \pm 0.5
P-Value	> 0.05	> 0.05	> 0.05	< 0.05	< 0.001

Experiment 2.

Addition of a daily supplement of 1450 mg RRR- α -tocopherol as topdressing to a diet already containing 300 mg *all-rac*- α -tocopheryl acetate and either 1.2 kg fatty acids from either sunflower or rapeseed cake gave a significant increase in both plasma and milk content of α -tocopherol. In contrast cows only fed 300 mg α -tocopheryl acetate and the highly unsaturated sunflower oil showed decreasing concentrations of α -tocopherol in plasma and milk during the five week experimental period (Figure 1 & 2).

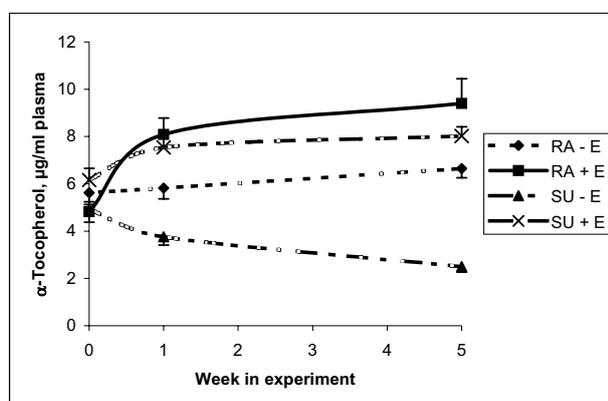


Figure 1. Plasma concentration of α -tocopherol in cows fed either with or without a daily supplement of 1450 mg RRR- α -tocopherol on top of diets containing 300 mg *all-rac*- α -tocopheryl acetate and sunflower or rapeseed cake (n = 10; means \pm SEM)

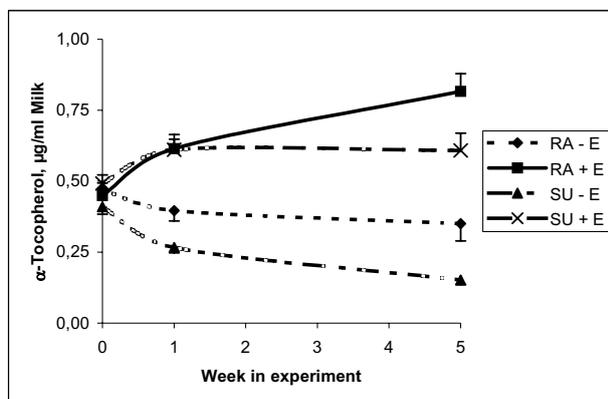


Figure 2. Milk concentration of α -tocopherol in cows fed either with or without a daily supplement of 1450 mg RRR- α -tocopherol on top of diets containing 300 mg *all-rac*- α -tocopheryl acetate and sunflower or rapeseed cake (n = 10; means \pm SEM)

Thus after five weeks on the experimental diets milk from cows fed RRR- α -tocopherol contained 0.47 $\mu\text{g/g}$ α -tocopherol more than milk from unsupplemented cows – irrespective of the fat source.

Experiment 3

Intramuscular injection of 2.5 g *all-rac*- α -tocopheryl acetate to cows showed that the 2S stereoisomers had the fastest disappearance from blood and milk. The synthetic 2R stereoisomers were retained longer than the 2S stereoisomers, but the natural RRR- α -tocopherol was retained in plasma for the longest time and also secreted into milk at the highest concentration (Table 2 and 3).

A consequence of this difference in turnover rate of the different stereoisomers of α -tocopherol in plasma is seen in Figure 3, where the relative distribution of α -tocopherol stereoisomers in plasma from cows fed a daily dose of 3000 mg *all-rac*- α -tocopheryl acetate for 16 days and compared with rats fed 1 mg *all-rac*- α -tocopheryl acetate for 10 days. From this figure it is seen that cows apparently discriminate in favour of the natural isomer to a much higher degree than rats.

Table 2. Plasma concentration and distribution of α -tocopherol stereoisomers after i.m. injection of 2.5 g *all-rac*- α -tocopheryl acetate to cows (n = 4; means)

	α -Tocopherol $\mu\text{g/ml}$ plasma	Relative distribution of stereoisomers, %				
		2S	RSS	RRS	RSR	RRR
Initial	3.0	1.7	2.2	2.3	1.6	92.2
1 days	16.9	24.3	15.1	15.1	13.5	32.0
5 days	5.1	2.5	10.5	12.6	9.9	64.5
10 days	4.1	0.5	4.7	6.3	4.2	84.4

Table 3. Milk concentration and distribution of α -tocopherol stereoisomers after i.m. injection of 2.5 g *all-rac*- α -tocopheryl acetate to cows (n = 4; means)

	α -Tocopherol $\mu\text{g/ml}$ plasma	Relative distribution of stereoisomers, %				
		2S	RSS	RRS	RSR	RRR
Initial	0.47	2.2	2.2	10.1	1.2	84.3
1 days	1.84	12.1	11.9	14.5	10.9	50.6
5 days	1.04	3.3	10.9	13.7	8.6	63.6
10 days	0.62	2.4	2.8	8.8	4.9	81.1

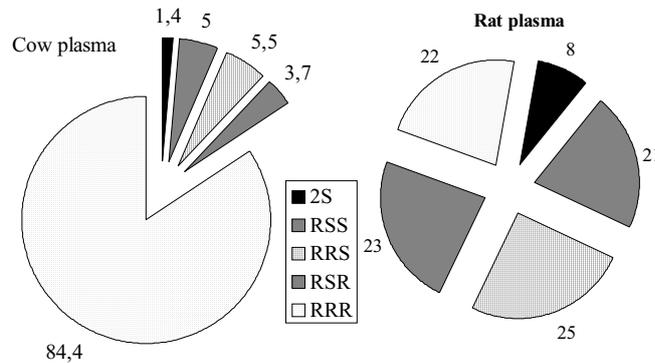


Figure 3. Relative distribution of α -tocopherol stereoisomers in plasma from cows fed 3000 mg *all-rac*- α -tocopheryl acetate daily for 16 days and rats fed 1 mg *all-rac*- α -tocopheryl acetate daily for 10 days (n = 5; means)

Summary

The content of vitamin E (α -tocopherol) in milk is dependent on the content in the cows feed. Thus cows' grassing fresh green pasture has a significant higher content of α -tocopherol in milk than cows fed corn silage or a high proportion of concentrate. Numerous studies have shown a poor transfer of synthetic α -tocopherol from feed to cows' milk. Thus only a small enrichment of the milk has been observed even though the cows' were fed more than 3 g *all-rac*- α -tocopheryl acetate per day. The present results shows that it is possible to increase α -tocopherol content of milk with about 0.5 $\mu\text{g/g}$ milk by addition of natural α -tocopherol as topdressing to the cows. Results on the fate of the different stereoisomers in plasma and milk from cows showed that cows favour the natural stereoisomer over the synthetic stereoisomers to a higher degree than previous reported for rats.

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Additional consumption and diet's supplementation participation in vitamins intake during alimentation of young female students of the Military University of Technology in Warsaw

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Introduction

The life style and especially nutrition manner are very important among number of factors influencing the society health state. Rational nutrition is particularly important during the development period i.e. in childhood and adolescence. Nutrition manner of students is a subject of number of researches recently carried out in Poland (Gacek, 2001; Kłos et al., 2004; Olędzka et al., 2001; Ostrowska et al., 2000; Seidler et al., 2000; Stopnicka et al., 2000; Wądołowska et al., 2000; Zielke et al., 2000).

As it results from the carried out researches nutrition of young people is not satisfactory. Among many shortcomings occurring in the students' nutrition the following should be mentioned: irregular alimentation characterized by repeated skipping certain meals as well as too low consumption of milk and dairy products, fruits, vegetables and dark bread etc. comparing to the recommendations. In case of women number of alimentation mistakes results from striving after perfect, slim figure. Results of some researches show occurrence of underweight among female students (Olędzka et al., 2001; Ostrowska et al., 2000; Zielke et al., 2000).

Women, candidates for professional soldiers, have been studying in Polish military academies for many years. They make a group of young persons coming from different social environments, people of different economic status and different nutritional habits and preferences. During the studies students are served four meals (breakfast I, breakfast II (lunch), dinner, supper). The poll conducted among female students of Military University of Technology (MUT) showed that some of them did not eat all the meals served during the day. At the same time they declare consumption of additionally bought food. It was found that diet's supplementation is quite common among female students (Rozmysł et al., 2004). Therefore it can be concluded that participation of additional consumption and diet's supplementation among these female stu-

dents may significantly affect the fulfillment of obligatory in Poland norms for particular nutritive elements including vitamins.

The aim of the work was estimation of additional consumption and diet's supplementation participation in the norms for vitamins fulfillment (on the safe level) for female students of MUT i.e. young women aged 19-25 physically active (Ziemiański et al., 1998).

Material and Methods

A poll regarding additional consumption and diet's supplementation among 84 female students of MUT in Warsaw was conducted in 2004. To calculate the vitamins content in additionally eaten food the software „DIETETYK 2” issued by the National Food and Nutrition Institute in Warsaw (Pakiet, 2001) was used. The following matters were taken into account in the researches: frequency of particular meals consumption in the canteen during the week, level of selected vitamins derived from additionally eaten food and diet's supplementation and differentiation of additional consumption and supplementation participation in fulfillment of norm for vitamins for young women, obligatory in Poland, was established as well.

Results

The average age of examined women was 22.9 ± 1.0 . Before studies 55.9% of examined lived in the cities, 38.1%, came from the country and 6.0% from small towns.

Up to 92.9% of examined ate meals, with different frequency, in the canteen while 7.1% ate mainly at home. All the persons eating in the canteen bought additional food products.

The most frequently eaten were: sweets and confectionery products, fruits, fruit juices, mineral waters and yogurts etc.

It was found that frequency of eating meals in the canteen was distinctly diversified (Tab.1.).

It should be underlined that considerable percentage of examined women did not eat certain meals in the canteen. The biggest number of examined students did not eat breakfasts II (lunches) (64.1%) and suppers (28.2%).

Table 1. Frequency of meals eaten in the canteen during the week

Kind of meals	Participation of examined (%)				
	Particular meals consumption frequency				
	Every day	5-6 days a week	3-4 days a week	1-2 day a week	Never
Breakfast I	10.3	55.1	11.5	9.0	14.1
Breakfast II (lunch)	6.4	19.2	6.4	3.9	64.1
Dinner	11.5	38.5	29.5	6.4	14.1
Supper	6.4	16.7	25.6	23.1	28.2

Table 2. Participation of additional consumption and diet's supplementation in fulfillment of recommended in Poland norms for vitamins*

Vitamins	Average daily content derived from additional consumption and diet's supplementation	Participation in fulfillment of recommended norms * (%)
Vitamin A [µg retinol equivalents]	708.1 ±1104.3	118.0
Vitamin E [mg α-tocopherol equivalents]	13.0 ±28.1	162.5
Thiamine [mg]	1.02 ±1.48	56.7
Riboflavin [mg]	1.13 ±1.35	56.5
Pyridoxine [mg]	1.67 ±2.89	83.5
Folic acid [µg]	142.2 ±177.8	54.7
Ascorbic acid [mg]	124.3 ±129.4	207.2
Niacin [mg niacin equivalents]	7.5 ±14.1	37.5
Vitamin B ₁₂ [µg]	3.0 ±11.5	150.0

* Recommended daily norms for vitamins (safe level) for women aged 19-25 physically very active (Ziemlański et al., 1998).

Carried out researches revealed that 67.9% of students eating in the canteen supplemented the diet. Vitamins and multicomponent products containing vitamins predominated among used supplements. Up to 64.1% students used such supplementation.

As it results from carried out examinations additional consumption including supplementation, delivered vitamins in amounts exceeding the values recommended in the norms obligatory in Poland.

Average daily intake of such vitamins as A, E, B₁₂ and C was as follows: 708.1±1104.3 µg, 13.0±28.1 mg, 3.0±11.5 µg, 124.3±129.4 mg, what made 118.0%, 162.5%, 150.0% and 207.2% of recommended norms respectively (table 2).

Furthermore additional food consumption and diet's supplementation significantly affected the fulfillment of Polish nutrition recommendations for other examined vitamins such as B₁, B₂, B₆, niacin and folic acid. Average daily intake of m/a vitamins made from 37.5% for niacin up to 83.5% for vitamin B₆, of recommended norm. Significant individual differentiation in additional consumption and supplementation participation in fulfillment the norm for vitamins was found.

Table 3. Differentiation of additional consumption and supplementation participation in fulfillment of recommended in Poland norms for vitamins among examined group of female students of Military University of Technology in Warsaw

Vitamins	Recommended norms fulfillment degree (%)				
	≤ 25	26 – 50	51 - 75	76 - 99	100 i >
	Participation of examined (%)				
Vitamin A	41.0	12.8	7.7	3.9	34.6
Vitamin E	41.0	20.5	7.7	2.6	28.2
Thiamine	55.1	11.5	9.0	7.7	16.7
Riboflavin	48.7	14.1	6.4	9.0	21.8
Pyridoxine	46.2	14.1	6.4	10.2	23.1
Niacin	65.4	15.4	3.8	5.1	10.3
Folic acid	41.0	27.0	11.5	5.1	15.4
Ascorbic acid	18.0	9.0	5.1	7.7	60.2
Vitamin B ₁₂	44.9	12.8	10.2	9.0	23.1

The data presented in the table 3 shows that students whose vitamins intake from additional consumption and supplementation did not exceed 50% made the most numerous groups. The vitamin C which intake met the requirements, and even exceeded it, in most cases (60.2%) was an exception.

Additional consumption and diet's supplementation among examined female students of MUT are significant elements of norms for vitamins fulfillment and therefore should be included in nutrition researches.

Conclusions

1. Frequency of eating in the canteen among female students of MUT is significantly differentiated. Considerable percentage of examined did not eat certain meals in the canteen at all.
2. All students eating in the canteen bought additional food products and 67,9% of them supplemented the diet, including 64.1% supplementing it with vitamin products.
3. Additional consumption together with supplementation delivered number of vitamins (vitamin A, E, B₁₂, C) in amounts exceeding values contained in the norms obligatory in Poland (on the safe level)
4. Significant individual differentiation in additional consumption and supplementation participation in fulfillment of the norm for vitamins was found in the carried out researches.

Summary

A poll among 84 female students of MUT in Warsaw was conducted in 2004. Carried out examinations revealed that considerable percentage of students did not eat certain meals in the canteen. All students eating in the canteen bought additional food products and 67,9% of them supplemented the diet. Additional consumption together with supplementation delivered many vitamins (vitamin A, E, B₁₂, C) in amounts exceeding levels considered as safe in the norms obligatory in Poland as well as significantly influenced on fulfillment of recommendations for other examined vitamins. Considerable individual differentiation in additional consumption and supplementation in norms for vitamins fulfillment was found.

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Does dietary intake of rosehip puree affect levels of carotenoids, vitamin E and vitamin C in human plasma?

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Introduction

During the last years, the use of some wild fruits as raw materials for a lot of products increased. Rosehips as fruits of wild roses are used to produce puree, jam and juice. Rosehips have traditionally been used as a vitamin supplement or for health food production, as the fruits are a rich source of ascorbic acid. Studies have also shown that rosehip extract inhibits lipid oxidation in vitro and reduces the chemotaxis and chemoluminescence of leucocytes (Winter et al. 1997, Larsen et al. 2003). Extracts also possess antioxidative capacity as well as antimutagenic effects (van Rensburg et al. 2005). The anti-inflammatory properties of rosehips are useful as a natural treatment in patients with osteoarthritis (Winter et al. 1999, Rein et al. 2004). Until now it is not clear which compounds are responsible for these effects.

In addition to ascorbic acid, rosehips deliver other antioxidants such as carotenoids, tocopherols/tocotrienols and phenolics. Fruits of wild roses showed remarkable contents of lycopene with an unexpected isomer pattern (Böhm et al. 2003). Lycopene is an acyclic carotenoid which is found only in few foods such as tomatoes and tomato products. Recent epidemiological studies showed that diets rich in lycopene were inversely correlated with the risk of many chronic diseases, such as cancer and heart diseases (Giovannucci 1999, Rissanen et al. 2002). All these properties can only be effective in vivo if lycopene and the other ingredients are available to the human organism.

The purpose of the study was to determine the effect of dietary intake of rosehip puree on plasma levels of lycopene. The relationships between rosehip consumption and plasma levels of other carotenoids, vitamin C and vitamin E were explored, too.

Materials and Methods

Five subjects (3 women and 2 men) ranging from 20 to 24 years with a body mass index (BMI) between 19 and 26 kg/m² participated in the study. The participants were non-smokers and did not take carotenoid, vitamin A, vitamin E or vitamin C supplements. Informed written consent was obtained from each participant and the protocol was approved by the Local Ethical Committee. All persons avoided food rich in lycopene like tomatoes and tomato products, water melons, yellow and red pepper, pink grapefruit, papayas, apricots, guavas, rosehip products and sea buckthorn products for a two weeks depletion period and the following four weeks of intervention. After the depletion period, the five participants ingested 38 g rosehip puree combined with 2.5 g sun flower oil twice a day for four weeks. Fasting blood samples were withdrawn from the participants prior the study and thereafter weekly. The blood samples were centrifuged and aliquots of plasma were stored at –80 °C until analysis.

Vitamin C was determined spectro-photometrically according to the method of Speitling et al. (1992). Carotenoids were extracted according to Bieri et al. (1985) and analysed using a C₃₀-HPLC-method according to Böhm (2001). Vitamin E compounds (tocopherols and tocotrienols) were analysed by high performance liquid chromatography (Balz et al. 1992).

Results are expressed as means ± standard deviations. Differences between variables were tested for significance by using the one-way ANOVA procedure (Tukey) for the basal values and for all other results the general linear model (GLM) for the two-way ANOVA procedure (SPSS for Windows, Release 10.07 (June 2000, SPSS Inc., Chicago)), using a level of significance of $p < 0.05$.

Results and Discussion

Consumption of 76 g rosehip puree and 5 g sun flower oil delivered: 31 mg vitamin C, 5 mg lycopene, 1 mg β-carotene and 8.4 μmol vitamin E (sum of tocopherols and tocotrienols).

The plasma levels of vitamin C are shown in **Figure 1**. After the two weeks diet with low lycopene intake, the vitamin C concentration of all participants significantly decreased ($p < 0.05$) due to reduced consumption of fruits and vegetables with high

vitamin C contents. Vitamin C contents were significantly enhanced ($p < 0.05$) relative to the depleted state from $426 \pm 40 \mu\text{mol/L}$ to $540 \pm 95 \mu\text{mol/L}$ after one week of supplementation with rosehip puree. The plasma levels remained nearly stable during the next three weeks of supplementation. Increase of vitamin C in plasma during the intervention phase indicated a good bioavailability of vitamin C from rosehip puree.

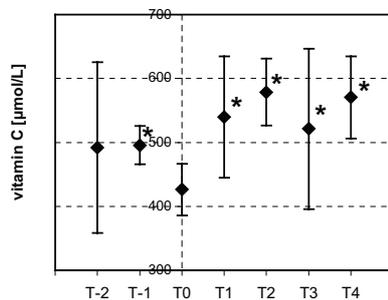


Figure 1: Plasma contents of vitamin C (mean values and standard deviations) over time in subjects consuming daily portions of rosehip puree for four weeks after a two weeks depletion period, * significantly different to T0 ($p < 0.05$)

Representative HPLC chromatograms of the carotenoid separation in plasma extracts are presented in **Figure 2**. The following carotenoids were detected in plasma of the participants: lutein, zeaxanthin, canthaxanthin, β -cryptoxanthin, α -carotene, β -carotene, rubixanthin and lycopene.

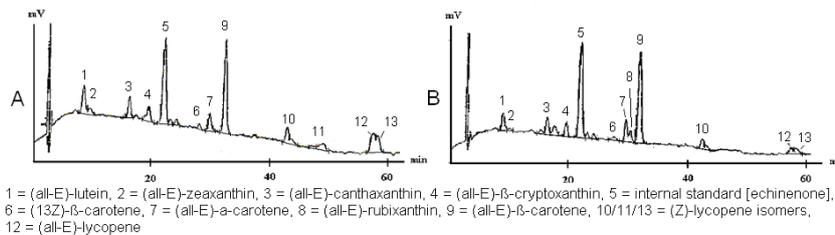


Figure 2: HPLC chromatogram of human plasma extracts before (A) and after (B) supplementation with rosehip puree, C_{30} (250 x 4.6 mm, 5 μm) column (Trentec, Germany), 1.3 mL/min, gradient of methanol and MTBE, 17 $^{\circ}\text{C}$, 470 nm

After the two weeks diet with low lycopene intake, the total-lycopene plasma concentration decreased ($p < 0.05$) from $0.54 \pm 0.19 \mu\text{mol/L}$ to $0.26 \pm 0.15 \mu\text{mol/L}$. Other publications reported comparable plasma lycopene clearance rates (Allen et al. 2003,

Böhm and Bitsch 1999). Surprisingly, an increase of the plasma lycopene was not observed during the supplementation with rosehip puree. Total-lycopene decreased significantly ($p < 0.05$) to $0.20 \pm 0.07 \mu\text{mol/L}$ after the four weeks intervention period. Results are shown in **Figure 3**. This study showed that lycopene from the consumed rosehip puree was not bioavailable. In contrast, the consumption of tomatoes, tomato products or lycopene capsules (Böhm and Bitsch 1999), of watermelons (Edwards et al. 2003) or of gac fruits (Vuong et al. 2002) increased the human plasma levels of lycopene. Higher intestinal absorption of lycopene from processed tomato products compared to unprocessed tomatoes was described in different studies (Gärtner et al. 1997, Porrini et al. 1998, van het Hof et al. 2000). Detailed information about production of the consumed rosehip product is not known and therefore it may be assumed that heating of the puree is needed to increase the lycopene bioavailability due to disruption of the food matrix and the subsequent release of lycopene from this matrix and from carotenoid-protein complexes (Britton 1995).

Contrary to lycopene, a good bioavailability of rubixanthin was reflected in the significant increase ($p < 0.05$) of the plasma levels after the consumption of the rosehip puree. Results are shown in **Figure 3**. Rubixanthin was not detected in human plasma at the beginning of the intervention and increased significantly ($p < 0.05$) to $0.02 \pm 0.02 \mu\text{mol/L}$ after one week and to $0.08 \pm 0.04 \mu\text{mol/L}$ after four weeks of supplementation with rosehip puree. Rubixanthin is a specific carotenoid of the fruits from wild roses and not present in other foodstuffs. Therefore, a daily consumption of rosehip puree with low content of rubixanthin ($<$ limit of quantitation) affected levels of rubixanthin in human plasma.

Regarding other plasma carotenoids (lutein, zeaxanthin, canthaxanthin, cryptoxanthin, α -, β -carotene), no significant differences were observed in plasma contents during the entire study period (data not shown).

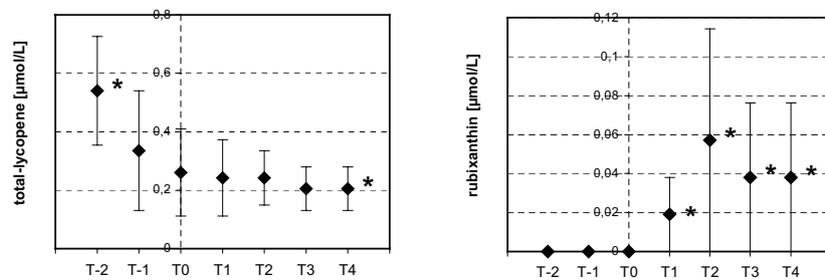


Figure 3: Plasma contents of total-lycopene and (all-E)-rubixanthin (mean values and standard deviations) over time in subjects consuming daily portions of rosehip puree for four weeks after a two weeks depletion period, * significantly different to T0 ($p < 0.05$)

The contents of tocopherols and tocotrienols in plasma did not change significantly ($p > 0.05$) during the depletion period and were not affected by four weeks of supplementation with rosehip puree (data not shown). This might be explained by the fact that the vitamin E intake was not controlled during the study.

Summary

In conclusion, ingestion of 38 g rosehip puree combined with 2.5 g sun flower oil twice a day for four weeks resulted in significantly increased plasma concentrations of vitamin C and significantly increased levels of rubixanthin, a specific carotenoid of rosehips. The plasma levels of lycopene and other plasma carotenoids, tocopherols and tocotrienols were not affected by four weeks of supplementation with rosehip puree. Further investigations will clarify the reasons of the low bioavailability of lycopene from rosehip puree.

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Mechanisms of the insulin sensitising effect of high supranutritional selenate doses in type II diabetic dbdb mice

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In recent years a fascinating physiological aspect has been found for selenate. Selenate administration in supranutritive doses (daily administration of amounts up to the individual LD₅₀ for about 8 weeks) to rats with streptocotozin induced type I diabetes led to a sustained correction of their diabetic status including the decrease of the elevated blood glucose concentration and considerable changes in the expression of abnormally expressed glycolytic and gluconeogenic marker enzymes (1, 2, 3, 4, 5, 6, 7). From *in vivo* experiments and *in vitro* studies with tissue cultures it was concluded that enhanced phosphorylation reactions at the β subunit of the insulin receptor and further components of the insulin signalling cascade are responsible for the so-called "insulinomimetic properties" of selenate (8, 9). The oral treatment of mice with alloxan induced type I diabetes with a high dose of selenite (4 mg/kg body weight and day) failed to reduce hyperglycemia in these animals. This seems to be based on differences in the intermediary metabolism of selenite and selenate (10). Insulinomimetic properties of selenate could also be found in type II diabetic dbdb mice. In this animal model featuring severe symptoms of type II diabetes (11, 12), the antidiabetic effect of selenate could be attributed to the reduction of insulin resistance whereas the *in vivo* administration of selenite did not result in a significant amelioration of insulin resistance and diabetes (11).

The present study with young female dbdb mice was therefore carried out to investigate the mechanisms by which selenate influences insulin resistance and metabolic pathways in type II diabetic mice.

Materials and Methods

Animals and diets

Twenty-one young female dbdb mice (C57BL/KsOlaHsd-Leprdb) aged 6 weeks with an average body weight of 43.7±2.03 g were randomly assigned to 3 groups of 7 animals each (selenium deficient = 0Se, selenite treated group = SeIV and selenate treated group = SeVI) The animals of all groups were fed a selenium deficient experimental diet (<0.020 mg Se/kg diet) based on torula yeast. Mice of the group 0Se were kept on selenium deficiency for eight weeks. The animals of the groups SeIV and SeVI were supplemented with amounts increasing from 15% up to 35% of the LD₅₀ of sodium selenite and sodium selenate by week 8 in addition to the diet by tube feeding (LD₅₀ of sodium selenite or of sodium selenate ~ 3.5 mg/kg body weight).

Performance of a whole body insulin sensitivity test (IST)

Before subjecting the mice to the specified dietary conditions (initial status) and after 8 weeks under experimental conditions their whole body insulin resistance was evaluated. Insulin sensitivity tests (ISTs) in mice fasted overnight were performed by subcutaneous injection of 2 I.U. insulin/kg body weight (Insuman® Infusat 100 I.U./mL from AVENTIS Pharma Deutschland GmbH, Frankfurt/Main) and subsequent determination of glucose concentration

(30, 60, 90, 120, 180, 240 and 300 minutes) in blood sampled from the tail vein using a glucometer (Bayer Elite).

Determination of biochemical and physiological parameters

a) Determination of the activity of cytosolic protein tyrosine phosphatases (PTPs) in the liver and assay of the „in vitro inhibition“ of PTPs by different selenium compounds

PTP activity was determined with modifications according to a method based on the hydrolysis of paranitrophenyl phosphate (pNPP) [12, 13, 14].

b) RT-PCR analysis to examine the expression of protein tyrosine phosphatase 1B (PTP1B) and peroxisome proliferator activated receptor gamma (PPAR γ)

For the 2-step RT-PCR analysis of PTP1B-and PPAR gamma-expression in the liver total RNA was prepared using the acid guanidinium thiocyanate extraction (15). 3 RNA-pools from 2 animals were prepared. Five μ g of each RNA-pool were reverse transcribed (RevertAID™ H Minus First Strand cDNA Synthesis Kit, #K1631 from MBI Fermentas).

Gene	Length of amplicate	Forward and reverse primer	Annealing temperature	Number of amplification cycles (x-times)
PTP1B	701	5'-GAT GGA GAA GGA GTT CGA GGA G-3' 5'-CCA TCA GTA AGA GGC AGG TGT C-3'	59.2	30
PPAR γ	348	5'-GAG TCT GTG GGG ATA AAG CAT C-3' 5'-CTC CAG GAC TCC TGC ACA T-3'	57.6	31
GAPDH	303	5'-ACG GGA AGC TCA CTG GCA TG-3' 5'-CCA CCA CCC TGT TGC TGT AG-3'	c.f. gene specific temperatures	26

The PCR reactions for the amplification of fragments from the coding sequence of the genes examined were carried out in a reaction volume of 50 μ L using the primer pairs shown above. The amplification products were separated by electrophoresis in 1.5% agarose gels containing 0.1 μ g/mL ethidium bromide and optical density was evaluated using the software for the Syngene Imager. The expression of the genes examined was normalised to GAPDH expression. Additionally the expression of the above mentioned genes was also examined in RNA samples obtained from 3 age- and sex-matched non diabetic black 6 mice.

c) Measurement of parameters of lipid metabolism

Crude lipids were extracted from 1:10 (w/v) homogenates prepared in 0.154 mol/L NaCl using a hexane:isopropanol (3:2) mixture containing 0.005% butylated hydroxytoluene. After centrifugation and evaporation of the solvent lipid concentration was determined gravimetrically and the lipids were resolved in 1 mL hexane and frozen at -20° C until further analysis. The concentration of triglycerides, cholesterol and phospholipids in liver lipid extracts were determined with the test kits Biocon, Bangalore, India (Fluitest®TG), Biocon, Bangalore, India (Fluitest®CHOL) and Boehringer, Mannheim, Germany. The protein content in liver homogenates was determined using a standard protocol (16).

Results

Whole body insulin sensitivity

In selenium deficient mice initial blood glucose concentration before insulin injection (time 0 minutes) was 1.5 to 2 times higher than in the initial status and in the selenate treated mice. The lowering of blood glucose concentration in these groups takes place at significantly higher mean blood glucose concentrations. The higher insulin resistance in selenium deficient and selenite treated mice is indicated by a steep rise in the blood glucose response curve towards the initial values (before insulin injection) after 120 minutes.

Activity of PTPs as important antagonists of insulin signaling

Selenate treatment for 8 weeks inhibited the activity of PTPs as important antagonists of insulin signaling by about 50% (0.58 ± 0.15 U/mg protein) as compared to selenium deficient (0.93 ± 0.23) and selenite (1.20 ± 0.36) treated animals.

Parameters of fatty acid metabolism

Treatment of dbdb mice with selenite increased total liver lipid content per gram fresh matter in tendency whereas selenate led to a significant increase of total liver lipids as compared to selenium deficiency. No changes could be found for the content of phospholipids (table 1).

Table 1: Parameters of lipid metabolism: Total lipids, triglycerides, phospholipids and cholesterol in the liver of dbdb mice treated with selenate for 8 weeks in comparison to mice on selenium deficiency and selenite treated mice based on one gram of liver fresh matter and one gram of total lipids, respectively

Parameter of liver fatty acid metabolism	0 Se	Se IV	Se VI
Parameters referring to one gram of liver fresh matter			
Total lipids (mg/g fresh matter)	$79.6 \pm 7.14^{a(<0.1)}$	99.4 ± 16.4^b	145 ± 26.9^c
Triglycerides (mg/g fresh matter)	36.3 ± 22.1^a	$62.7 \pm 10.6^{b(<0.1)}$	$71.0 \pm 24.0^{b(<0.1)}$
Phospholipids (mg/g fresh matter)	18.7 ± 1.19^a	18.6 ± 2.16^a	19.0 ± 1.32^a
Cholesterol (mg/g fresh matter)	3.30 ± 0.64^a	4.87 ± 1.00^b	3.35 ± 1.13^a
Parameters referring to one gram of liver lipids	0 Se	Se IV	Se VI
Triglycerides (mg/g lipids)	633 ± 80.9^a	639 ± 97.4^a	$491 \pm 150^{b(<0.1)}$
Phospholipids (mg/g lipids)	236 ± 20.8^c	190 ± 31.5^b	138 ± 35.7^a
Cholesterol (mg/g lipids)	43.6 ± 7.25^b	50.3 ± 11.91^b	24.1 ± 10.24^a
Rest to one gram lipids (mg)	87.4	120.7	346.9

Significant differences ($p < 0.05$) within a line are indicated by different superscripts, (< 0.1) shows a trend

A significantly higher cholesterol concentration was evident in selenite treated mice in comparison to selenium deficient and selenate treated mice.

When the lipid parameters were referred to one gram of total lipids all parameters measured were significantly lower in selenate treated mice in comparison to selenium deficient and selenite treated animals, whereas the remnant to one gram of total lipids was significantly increased by selenate treatment, presumably indicating an increased concentration of free fatty acids.

With regard to gene expression of genes related to insulin resistance, glucose metabolism and fatty acid metabolism some marked changes could be measured (figure 1 A and B). Selenium supplementation with selenite or selenate increased the expression of PTP1B, an important tyrosine phosphatase discussed in the context of insulin resistance, about 2- or 2.5fold in comparison to selenium deficiency. The expression of the peroxisome proliferator activated receptor gamma (PPAR γ) as an efficient target in the treatment of obesity and insulin resistance, which is mainly expressed in adipose tissue but also in the liver of obese rodents, was about 2.5fold increased in the liver of selenate treated mice in comparison to their selenium deficient and selenite treated companions. Under the conditions examined (up to 31 amplification cycles) no expression of PPAR γ could be detected in non diabetic black6 mice.

Expression of genes related to insulin resistance and fatty acid metabolism

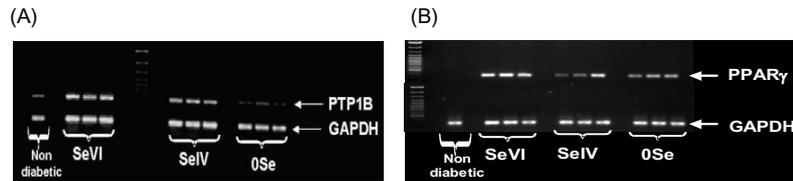


Figure 1 A and 1 B: Expression of PTP1B [A] and PPAR [B] in the liver of db/db mice treated with selenate for 8 weeks in comparison to mice on selenium deficiency, mice treated with selenite and non-diabetic black6-control mice relative to their respective GAPDH expression

Discussion

In the present study treatment of the db/db mice with supranutritional selenate doses effected an improvement of whole body insulin sensitivity in comparison to selenium deficient and selenite treated mice.

Activity and expression of PTPs as important antagonists of insulin signalling and particular changes in glucose metabolism

A significant decrease of PTP activity in the liver was obtained only by oral selenate administration. Supranutritional doses of both selenite and selenate increased the expression of PTP1B. There is no doubt that diabetic symptoms can be efficiently reduced by treatment with PTP1B enzyme inhibitors or antisense oligonucleotides, which reduce the mRNA expression and the protein synthesis of the enzyme (17, 18, 19, 20).

Explanations of the mechanism for reversible and irreversible PTP1B inhibition by glutathionylation in the presence of high concentrations of oxidized glutathione or formation of sulphenic,- sulphinic,- and sulphonic acid derivatives in the presence of hydrogen peroxide have been given involving the blocking as well as the stepwise oxidation of the cysteine SH-group of the active. Even in vivo an insulin dependent release of hydrogen peroxide in tissues leads to an oxidation of PTP1B and an increase in insulin signalling. (21, 22, 23, 24). From the results of this study and from further unpublished results we conclude that glutathionylation of PTPs due to a high GPx1 activity and a shift of the glutathione redox pair to a more oxidized state is the driving force for the increased expression of PTP1B.

Our results on PTP activity and expression can be interpreted as follows (figure 2): High supranutritional selenium doses effect a shift in the glutathione-redox system to a more oxidized state (25). An enhanced glutathionylation of PTPs is presumably the stimulus for an increase in gene expression.

Only in the case of very high selenate doses the inhibitory effect of intermediary selenate metabolites compensates for the increased expression which in turn leads to a correction of insulin sensitivity.

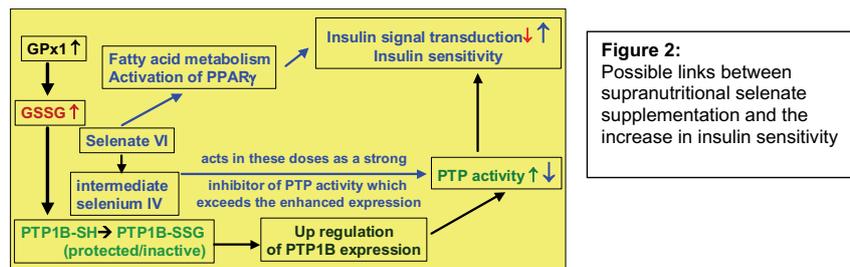


Figure 2: Possible links between supranutritional selenate supplementation and the increase in insulin sensitivity

Influence on fatty acid metabolism

In fatty acid metabolism supranutritional selenate leads to a significant decrease of plasma cholesterol and triglycerides. Concomitantly a significant increase in total liver lipid- and triglyceride concentration as well as in the expression of PPAR_γ was measured.

Peroxisome proliferator-activated receptors (PPARs) are originally transcription factors belonging to the superfamily of nuclear receptors. They act on DNA response elements as heterodimers with the nuclear retinoic acid receptor. Their natural activating ligands are fatty acids and lipid-derived substrates. Peroxisome proliferator activated receptor gamma (PPAR_γ) is considered to be one of the master regulators of adipocyte differentiation. The isoform PPAR_γ2 is abundantly expressed in mature adipocytes and is elevated in animals with fatty livers. The natural activating ligands of PPARs are fatty acids and lipid derived substrates. Thiazolidinediones were developed as antidiabetic drugs acting as synthetic ligands of PPARs. They increase peripheral glucose utilisation and reduce insulin resistance (26, 27). The whole complex of tissue specific actions and interactions of PPARs is not yet fully understood. In a study with transgenic mice, animals without liver PPAR_γ but with adipose tissue developed fat intolerance, increased adiposity, hyperlipidemia and insulin resistance. Thus it was concluded that liver PPAR_γ regulates triglyceride homeostasis, contributing to hepatic steatosis, but protecting other tissues from triglyceride accumulation and insulin resistance (28, 29).

Our study shows that a similar mechanism seems to be activated by selenate (figure 2). Selenate treated mice showed far less body weight gain and obesity than their selenium deficient and selenite treated companions they showed reduced plasma lipids and a distinct increase in liver lipids. The lipid fractions per gram of total lipids indicate that in selenate treated dbdb mice the “rest” to one gram of total lipids is significantly higher in comparison to both other groups and therefore demonstrate a higher amount of “Non-Esterified Fatty Acids”. These “Non-Esterified Fatty Acids” in turn can act as natural ligands of PPAR γ and therefore contribute to an increase in whole body insulin sensitivity.

Conclusion

The results of our study with type II diabetic dbdb mice give some new insight into the mechanisms by which the administration of supranutritional selenate influences diabetes and insulin resistance. One mechanism of interest is the inhibition of protein tyrosine phosphatases by intermediary selenate metabolites. This aspect of an antidiabetic action is closely linked to selenium metabolism. Furthermore we could demonstrate that the system of PPARs is also initiated by supranutritional selenate. The increased expression of liver PPAR γ presumably leads to a redistribution of whole body lipid stores resulting in an increase in liver lipids. In turn the concentration of lipids in the liver can provide natural ligands of PPAR γ and therefore contribute to the increase in insulin sensitivity.

Summary

To examine the mechanisms behind the insulin sensitising effect of high supranutritional selenate doses in a type II diabetic animal model 21 young female dbdb mice were randomly assigned to 3 experimental groups of 7 animals each. Group 0 was fed a selenium deficient diet for 8 weeks while the animals of groups Se IV (selenite) and Se VI (selenate) were supplemented with high supranutritional doses of selenite and selenate in addition to the diet.

- Selenate treatment of the mice increased whole body insulin sensitivity significantly in comparison to selenium deficiency and selenite treatment.
- Selenate administration to the mice (0.58 ± 0.15 U/mg protein) inhibited the activity of cellular protein tyrosine phosphatases (PTPs) in comparison to selenium deficient (0.93 ± 0.23) and selenite treated mice (1.20 ± 0.36).
- The application of supranutritional selenate doses resulted in a 2 to 3-fold increase in the expression of the peroxisome proliferator-activated receptor gamma (PPAR γ) accompanied by significant changes in total liver fat content and in the portions of triglycerides, phospholipides and cholesterol in comparison to both other groups.

The results of our study in dbdb mice showed that two efficient mechanisms, discussed as important factors for an increase in insulin sensitivity, were affected by the administration of high supranutritional selenate doses and therefore these mechanisms may have mediated the insulin sensitising effect of selenate.

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Literature cited

The literature is available with the author.

Effekte von Vitamin E und Vitamin C auf das zelluläre und humorale Immunsystem des Hundes

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Einleitung

Bei Vitamin C handelt es sich um eine wasserlösliche Verbindung, die an zahlreichen enzymatischen Reaktionen im Körper beteiligt ist und im intra- und extrazellulären Raum als Antioxidans wirkt (Bendich, 1990). Sie scheint für verschiedenste Komponenten des Immunsystems eine Bedeutung zu haben. In Leukozyten ist Vitamin C in hohen Konzentrationen vorhanden, während Infektionen wird es schnell umgesetzt und reduzierte Plasmagehalte sind oft mit verminderten Immunfunktionen verbunden (Hughes, 1999). Vitamin E ist ebenfalls ein wichtiges Antioxidans, das in den Membranen die Bildung von Lipidperoxiden hemmt (Meydani, 1995). Da Immunzellen einen hohen Gehalt an mehrfach ungesättigten Fettsäuren aufweisen, sind diese gegenüber oxidativem Stress besonders anfällig und auf eine ausreichende Versorgung mit Vitamin E angewiesen.

Der Bedarf einer Zuführung von Vitamin C besteht beim Hund nicht, da diese Spezies die Verbindung im Körper selber synthetisieren kann. Dennoch stellt sich die Frage, ob durch eine Zulage von Vitamin C über die Nahrung ein Effekt auf das Immunsystem hervorgerufen werden kann. Dieser Frage sollte bei gleichzeitiger hoher Versorgung mit Vitamin E in der vorliegenden Studie nachgegangen werden.

Material und Methoden

15 Hunde wurden randomisiert auf drei Gruppen aufgeteilt. Das Studiendesign entsprach einem modifizierten lateinischen Quadrat. Eine Gruppe erhielt 0 mg Vitamin C in Kombination mit 60 mg Vitamin E, die zweite 30 mg Vitamin C und 60 mg Vitamin E und die dritte 60 mg Vitamin C zusammen mit 60 mg Vitamin E pro Tag über 35 Tage. Das verabreichte Versuchsfutter wurde ohne Zusatz von Vitamin E und Vitamin C hergestellt. Nach einer siebentägigen Auswaschphase wurde die Behandlung

gewechselt, bis jede Gruppe die drei Kombinationen erhalten hatte. Vor und nach jeder Supplementierungsperiode fand eine Blutentnahme statt.

Blutuntersuchung: Im Blut der Hunde wurden biochemische und hämatologische Parameter untersucht. Außerdem wurde ein Differentialblutbild erstellt.

Durchflusszytometrie: Um die Lymphozytenpopulationen im peripheren Blut näher charakterisieren zu können, wurden monoklonale Antikörper (Serotec, Raleigh, USA) gegen die Oberflächenantigene CD4, CD8, CD21 und CD5 eingesetzt. Nach Markierung mit einem sekundären, fluoreszenzmarkiertem Antikörper (Southern Biotech. Ass., Birmingham, USA) wurde die gefärbten Zellen in einem Durchflusszytometer (FACSCalibur[®], Becton Dickinson, Franklin Lakes, USA) gemessen und anschließend prozentual ausgewertet.

Lymphozytenproliferation: Die proliferative Antwort von peripheren mononukleären Zellen (PBMCs) nach Stimulation mit 10 µg/ml Concanavalin A (ConA), 10 µg/ml Phytohämagglutinin (PHA) und 5 µg/ml Poke Weed Mitogen (PWM) wurde mit der 3H-Thymidin-Methode in Vollblutkultur gemessen.

IgA und IgG: Die Immunglobulin A- und G-Konzentration im Plasma wurde mittels kommerziell erhältlicher ELISA-Kits (Bethyl laboratories, Inc., Montgomery, USA) bestimmt.

Statistik: Als statistischer Test wurde eine einfaktorielle Varianzanalyse und nachfolgend der Test nach Scheffé durchgeführt. Um eine Veränderung der Werte vor und nach Supplementierung der jeweiligen Gruppen zu analysieren, wurde der gepaarte t-Test angewendet. Eine Signifikanz wurde bei $p < 0,05$ angenommen.

Ergebnisse

Blutuntersuchung: Die ermittelten hämatologischen und die biochemischen Werte des Blutes zeigten keine Abweichungen von den Referenzwerten. Eine Veränderung über den beobachteten Versuchszeitraum konnte für keine der verabreichten Supplementierungen beobachtet werden (Ergebnisse nicht dargestellt).

Durchflusszytometrie: Die Mittelwerte der prozentualen Anteile an CD4-positiven Lymphozyten lagen zwischen 47,9 und 50,9 % (Abbildung 1). Die Gesamtheit der Lymphozyten (CD5-positiv) lag im Mittel zwischen 81,4 und 83,2 %. CD8-positive kamen im Durchschnitt zu 17,2 bis 18,5 % vor. Der Anteil an B-Lymphozyten (CD21-positiv) unterlag den größten interindividuellen Schwankungen bei Werten zwischen

13,0 und 17,4 %. Insgesamt ergaben sich keine signifikanten Veränderungen des Anteils der untersuchten Lymphozytenpopulationen vor und nach der Supplementierung. Auch der Vergleich der verschiedenen Gruppen deckte keine signifikanten Unterschiede in Abhängigkeit von der Vitamingabe auf.

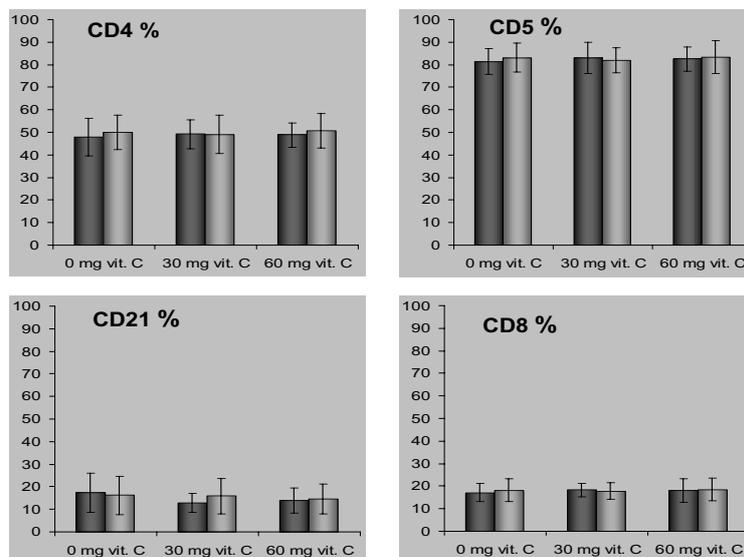


Abbildung 1: Prozentuale Anteile an CD4, CD8, CD21 und CD5-positiven Zellen nach Supplementierung der Hunde mit 0, 30 mg und 60 mg Vitamin C kombiniert mit 60 mg Vitamin E pro Tag (Mittelwert \pm Standardabweichung)

■ vor der Behandlung
 ■ nach der Behandlung

Lymphozytenproliferation: Die Stimulationsindizes (Wert stimulierter Zellen/ Wert unstimulierter Zellen) von Hunden nach Verabreichung von 30 mg Vitamin C über 35 Tage waren höher als vor der Behandlung (Abbildung 2). Dieses zeigte sich nach Stimulation mit allen drei Mitogenen und war für PHA und PWM signifikant. Bei Gabe von 60 mg Vitamin C wurde nach ConA-Stimulation ein signifikantes Absinken des Stimulationsindex beobachtet.

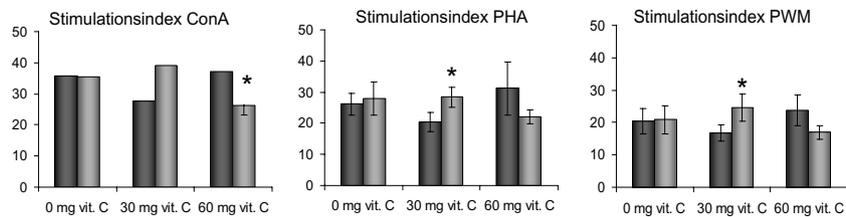


Abbildung 2: Stimmulationsindizes nach Stimulierung mit ConA, PHA und PWM nach Supplementierung der Hunde mit 0, 30 mg und 60 mg Vitamin C kombiniert mit 60 mg Vitamin E pro Tag (Mittelwert \pm Standardabweichung), *statistische Signifikanz ($p < 0,05$) im Vergleich von Ergebnissen vor und nach der Behandlung

■ vor der Behandlung
 □ nach der Behandlung

IgA und IgG-ELISA: Der Gehalt an IgA im Plasma veränderte sich während des Versuchs und zwischen den Gruppen statistisch nicht signifikant (Tabelle 1). Die IgG-Gehalte stiegen bei Vitamin C -Gabe gegenüber der Messung vor der Behandlung statistisch signifikant an.

Tabelle 1: IgA- und IgG-Gehalte im Plasma (mg/ml) nach Supplementierung der Hunde mit 0, 30 mg und 60 mg Vitamin C kombiniert mit 60 mg Vitamin E pro Tag (MW: Mittelwert, Std.: Standardabweichung), *statistische Signifikanz ($p < 0,05$) im Vergleich von Ergebnissen vor und nach der Behandlung

	Tag 0		Tag 35		
	MW	Std.	MW	Std.	
IgA	0 mg Vitamin C	1,34	0,81	1,67	1,66
	30 mg Vitamin C	1,15	0,55	1,38	0,96
	60 mg Vitamin C	1,63	1,43	1,35	1,14
IgG	0 mg Vitamin C	19,3	5,11	21,4	5,57
	30 mg Vitamin C	18,9	6,42	21,2*	4,35
	60 mg Vitamin C	19,9	5,91	26,1*	9,90

Zusammenfassung

Effekte von Vitamin E und Vitamin C auf das zelluläre und humorale Immunsystem des Hundes.

Bei Vitamin E und Vitamin C handelt es sich um für den Körper wichtige Antioxidantien, die in den intra- und extrazellulären Raum und in der Zellmembran ihre Wirkung entfalten. Durch einen Mangel an diesen Substanzen sowie durch einen erhöhten Umsatz kommt es zu oxidativem Streß mit negativen Auswirkungen auf das Immunsystem. Eine Supplementierung über den Bedarf hinaus führte bei verschiedenen Spezies zu einer gesteigerten Immunantwort. In der vorliegenden Studie sollte untersucht werden, ob durch eine Zulage von 0, 30 oder 60 mg Vitamin C, bei einer Spezies die in der Lage ist, Vitamin C zu synthetisieren, in Kombination mit einer hohen Aufnahme von Vitamin E (60mg/ Tag) ein Effekt auf das Immunsystem gezeigt werden kann. Zu diesem Zweck wurden 15 Beagles randomisiert auf drei Gruppen aufgeteilt und jeweils 35 Tage mit den drei verschiedenen Dosierungen behandelt. Veränderungen des Differentialblutbildes oder in der Zusammensetzung der Lymphozytenpopulation bleiben aus. Im Hinblick auf die Stimulierbarkeit von Lymphozyten zeigte sich, dass nach Behandlung mit 30 mg Vitamin C eine Steigerung auftrat, wohingegen es in Verbindung mit 60 mg Vitamin C zu einer Abnahme des Stimulationsindex kam. Der Immunglobulin G-Gehalt stieg in den mit Vitamin C versorgten Gruppen signifikant an. Es konnte in dieser Studie ein Effekt auf das Immunsystem anhand der gemessenen Parameter gezeigt werden. Weitere Studien werden notwendig sein, um die Wirkungsweise zu klären.

Summary

Effects of vitamin E and vitamin C on cellular and humoral immune parameters of dogs.

Vitamin C and vitamin E are important antioxidants in intra- and extracellular compartments and the cellular membrane. Oxidative reactions caused by a lack of antioxidants or an overproduction of free radicals can adversely affect many aspects of immune responses. A supplementation of these vitamins resulted in an immunostimulating effect in different species. In this study, ascorbic acid (0, 30 or 60mg /day) in combination with a high vitamin E (60mg /day) intake has been administered to dogs despite the fact that these animals are capable of synthesising this compound

to examine the effects on the immune system of adult dogs. For this reason 15 adult beagle dogs were randomly divided in three groups. Each group was administered the different supplementation, each for 35 days. Changes in the differential blood count or the expression of cell surface markers of lymphocytes were not found. Lymphocyte proliferation increased after treatment with 30 mg vitamin C and decreased after supplementation with 60 mg vitamin C per day. The IgG-concentration increased after administration of vitamin C in both dosages. In conclusion, a modification of two analysed immune parameters, lymphocyte proliferation and immunoglobulin concentration after treatment with vitamins C and E were shown. Further investigations are necessary to understand the mode of action on the immune system.

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Influence of feeding various amounts of minerals and vitamins to first lactating cows on some blood parameters

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Introduction

It is common practice to take blood from cows to monitor their health and nutritional condition. The measured values are compared with given reference values and if necessary, action can be taken to change the current state can be taken. This can, for example, be a variation in the feeding practices or treatments by a veterinarian. In the past many fallings below the reference values, especially for trace elements and vitamins, were reported in spite of recommended feeding. Because the recommendations of the GfE are relatively new (2001), the reference values were inspected more precisely. It was found that information about their origin or their age are very rare. Normally recommendations from the IFCC (1987) should have been used to determine reference values. This method is also described by Farver (1997). Furthermore the building of reference values depends on age, breed, season and feeding (Kraft, 1991, Stangassinger, 2003). Also the number of animals for the study is very important (Lumbdsen, 1978). Because this criteria could not be approved for the reference values used (Clinic for Cattle from the University for Veterinary Medicine Hannover), an experiment was carried out to monitor some blood values of cows, fed different amounts of vitamins and minerals.

Materials and Methods

In the present study, 30 German Holstein heifers were split into two feeding groups with 15 animals each. The cows were housed in a stable with computerized feeding automats for concentrates and computerized weighing troughs for roughage, allowing an automatic registration of individual feed intake. All animals received maize-grass-silage *ad libitum* at the rate of 60:40 (DM-base) and concentrate depending on their milk yield, so that the supply with energy and crude protein should not vary between the groups. The only difference between the groups was the amount of minerals and vitamins fed in the concentrate. Group 1 received mineral and vitamin levels according to the recommendations of the GfE (2001), whereas Group 2 was offered at least double the amount of minerals and vitamins. The duration of this experiment was the whole first lactation of the animals. During the experiment blood samples were taken

from the *vena jugularis* one week before calving and one, four, eight, 16 and 36 weeks after calving to monitor the blood values of all animals over the whole lactation period. The concentrations of calcium, magnesium, phosphorus, copper, zinc, β -carotene and Vitamins A and E in serum were analyzed (Cobas Mira®, Hoffmann La-Roche, Basel, Schweiz) by the Clinic for Cattle from the University of Veterinary Medicine in Hannover. Additionally feed and milk samples were taken and examined over the whole lactation period.

Results

As a consequence of the feeding regime, the daily mineral intake was significantly different between the groups (Table 1).

Table 1: Daily intake of minerals ($\bar{x} \pm s$) per 100 days of lactation

		Days 1-100	Days 100-200	Days 200-300
Ca (g/d)	Gr. 1	104,3 ^a ±24,9	114,8 ^a ±21,9	102,2 ^a ±22,2
	Gr. 2	279,8 ^b ±79,1	259,6 ^b ±53,7	208,0 ^b ±32,7
Mg (g/d)	Gr. 1	30,0 ^a ±6,9	33,6 ^a ±5,6	31,1 ±6,4
	Gr. 2	61,2 ^b ±15,9	58,6 ^b ±10,5	47,6 ±6,8
P (g/d)	Gr.1	73,0 ^a ±16,5	77,3 ^a ±13,3	67,8 ^a ±14,0
	Gr.2	117,1 ^b ±28,9	111,6 ^b ±19,2	89,6 ^b ±13,0
Cu (mg/d)	Gr. 1	182 ^a ±44,8	201,9 ^a ±37,1	168,8 ^a ±41,8
	Gr. 2	425,8 ^b ±128,4	406,9 ^b ±79,6	319,2 ^b ±53,4
Zn (mg/d)	Gr.1	952,3 ^a ±235,2	1292,2 ^a ±376,5	865,2 ^a ±286,7
	Gr. 2	2247,0 ^b ±690,1	2097,8 ^b ±456,5	1426,9 ^b ±251,4

a, b = significant differences between the group mean values of unequal superscripts (p<0,05)

At the same time there were no significant differences between the groups in their live weight (LW), their dry-matter-(DMI) and energy-intake (NEL) as requested (Table 2).

Table 2: Dry matter intake, energy intake and live weight ($\bar{x} \pm s$)

		Days 1-100	Days 100-200	Days 200-300
DMI (kg/d)	Gr. 1	15,8 ±3,5	17,5 ±2,9	16,2 ±3,4
	Gr. 2	16,9 ±3,4	17,9 ±2,8	16,2 ±2,7
NEL (MJ/d)	Gr. 1	113,3 ±24,9	125,1 ±20,5	113,4 ±23,6
	Gr. 2	117,2 ±24,1	123,0 ±19,3	109,6 ±17,5
LW (kg)	Gr. 1	568,3 ±41,8	601,2 ±42,6	620,8 ±47,2
	Gr. 2	566,6 ±44,0	592,9 ±46,2	609,5 ±46,8

The milk yield and fat content varied significantly between the groups, but the fat-corrected-milk (FCM) was compensated, shown in table 3. The differences in milk fat

content can not presently be explained. The other milk components (protein, lactose, urea) did not vary significantly between the groups.

Table 3: Milk and FCM-yield and fat content ($\bar{x} \pm s$)

		Days 1-100	Days 100-200	Days 200-300
Milk (kg/d)	Gr. 1	26,1 ±5,1	26,8 ^a ±4,5	22,4 ^a ±4,9
	Gr. 2	24,6 ±4,6	22,9 ^b ±3,2	18,9 ^b ±2,5
Fat (%)	Gr. 1	3,7 ^a ±0,9	3,7 ^a ±0,6	4,3 ^a ±0,7
	Gr. 2	4,3 ^b ±0,8	4,4 ^b ±0,6	4,8 ^b ±0,5
FCM (kg/d)	Gr. 1	24,8 ±5,3	25,2 ±4,2	23,3 ±5,0
	Gr. 2	25,5 ±4,2	24,2 ±3,2	21,1 ±2,6

a, b = significant differences between the group mean values of unequal superscripts ($p < 0,05$)

The results of the measured blood values are demonstrated for macro elements (Table 4), micro elements (Figure 1 and 2) and vitamins (Table 5). The serum concentrations of the macro elements calcium, magnesium and phosphorus showed no variations from the reference values as a consequence of their homeostasis. Only the phosphorus concentrations varied between the feeding groups, whereas the serum concentrations of the Group 2 were higher over the whole period. But these higher concentrations in Group 2 were also present before calving and were not statistically significant (Table 4). Increased serum phosphorus concentrations were also detected by Lopez (2003) after feeding higher amounts, whereas Mores et al. (1992) did not find differences in the blood serum by feeding different amounts of phosphorus.

The micro elements presented other relations. The serum copper concentrations of both groups were marginally under the minimum reference value for the most time. Only at one week after calving were the copper concentrations for both groups in the reference range of 12–24 $\mu\text{mol/l}$ (Figure 1). Engle et al. (2001) and Du et al. (1996) also showed equal serum copper concentrations when feeding different amounts of copper.

Table 4: Serum concentrations ($\bar{x} \pm s$) of some macro elements

		1. Week	1. Week	4. Week	8. Week	16. Week	36. Week
Ca 2,1-3 mmol/l	Gr. 1	2,40 ± 0,11	2,42 ± 0,17	2,47 ± 0,17	2,47 ± 0,14	2,46 ± 0,09	2,48 ± 0,13
	Gr. 2	2,43 ± 0,12	2,48 ± 0,20	2,48 ± 0,14	2,47 ± 0,15	2,42 ± 0,11	2,45 ± 0,11
Mg 0,7-1,2 mmol/l	Gr. 1	0,96 ± 0,10	0,87 ± 0,14	1,03 ± 0,12	1,01 ± 0,10	0,89 ± 0,12	0,93 ± 0,16
	Gr. 2	0,95 ± 0,13	0,93 ± 0,11	1,02 ± 0,11	1,03 ± 0,10	0,94 ± 0,10	0,95 ± 0,10
P 1,1-2,4 mmol/l	Gr. 1	1,54 ± 0,29	1,30 ± 0,27	1,44 ± 0,36	1,41 ± 0,29	1,41 ± 0,20	1,46 ± 0,24
	Gr. 2	1,63 ± 0,15	1,51 ± 0,21	1,61 ± 0,24	1,55 ± 0,24	1,59 ± 0,21	1,59 ± 0,22

Grey marked values are the reference values for the elements

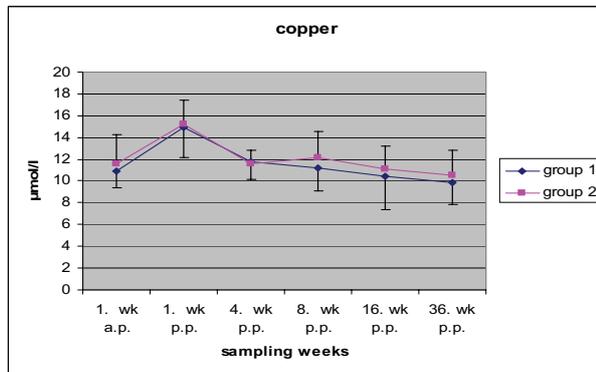


Figure 1: Serum copper concentrations ($\bar{x} \pm s$) over 36 weeks of lactation

The zinc concentrations in the serum illustrated differences between the groups. After calving, the zinc concentrations of Group 1 were lower than the reference limit and increased into the reference range only after Week 16, whereas the serum concentrations of Group 2 were in agreement with the reference interval (12-24 µmol/l, Figure 2) over the whole lactation period. This reaction of increased serum concentrations after feeding higher amounts of zinc are also reported by Kirchgessner et al. (1978, 1982).

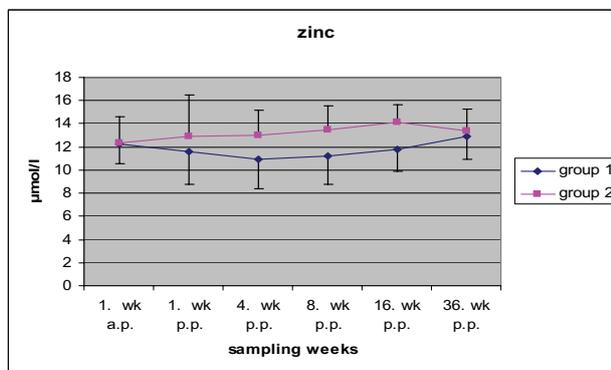


Figure 2: Serum zinc concentrations ($\bar{x} \pm s$) over 36 weeks of lactation

The results of the serum vitamin concentrations exhibited that the concentrations of β-carotene in the blood were lower than the reference values in both groups. Only at Week 36 were the concentrations in the reference range. The Vitamin A concentrations were not significantly different between the groups, but there were significant

variations of the serum concentrations of Vitamin E between the groups. At Weeks 16 and 36, the Vitamin E concentrations of Group 2 were significantly higher. This increase of the serum concentration after feeding higher amounts of Vitamin E was also described by Weiss et al. (1994) and Brzezinska-Slebodzinska et al. (1994).

Table 5: β -carotene-, Vitamin A and E-concentrations ($\bar{x} \pm s$) in the serum

	1. Week a.p.	1. Week p.p.	4. Week p.p.	8. Week p.p.	16. Week p.p.	36. Week p.p.
β-carotene Gr. 1	174 \pm 31	153 \pm 21	147 \pm 21	150 \pm 25	196 \pm 43	220 \pm 37
>200 μ g/dl Gr. 2	175 \pm 41	159 \pm 36	146 \pm 24	168 \pm 43	186 \pm 31	203 \pm 44
Vitamin A Gr. 1	0,57 \pm 0,22	0,61 \pm 0,17	0,70 \pm 0,17	0,72 \pm 0,15	0,72 \pm 0,15	0,80 \pm 0,15
>0,3 mg/l Gr. 2	0,54 \pm 0,14	0,53 \pm 0,12	0,67 \pm 0,16	0,69 \pm 0,15	0,80 \pm 0,24	0,75 \pm 0,21
Vitamin E Gr. 1	3,18 \pm 0,46	3,14 \pm 0,93	3,95 \pm 1,15	5,28 ^a \pm 1,26	7,19 ^a \pm 1,95	8,73 \pm 2,21
>3,0 mg/l Gr. 2	3,46 \pm 0,73	3,23 \pm 0,86	4,67 \pm 1,27	6,83 ^b \pm 1,19	8,54 ^b \pm 1,75	8,63 \pm 2,11

a, b = significant differences between the group mean values of unequal superscripts ($p < 0,05$)

Grey marked values are the reference values for the elements

Conclusions

When feeding minerals and vitamins like recommended by the GfE (2001), there were no observed clinical deficiency syndromes, but the measured blood concentrations of micro elements and vitamins were not in agreement with the given reference values. In spite of recommended feeding of first lactating cows (Group 1), the concentrations of copper, zinc and β -carotene in serum were lower than the given reference range. Also by feeding double amounts of vitamins and minerals (Group 2) over the whole lactation period, the serum concentrations of copper and β -carotene were under the lower limit of the reference value. When feeding double the recommended amounts of minerals and vitamins there were only significantly higher serum levels for Zn and Vitamin E. Further investigations, in consideration of other indicator samples (liver, hair, etc.), are necessary to prove, whether serum alone is a qualified medium for analyzing these minerals (especially for copper) and vitamins. Then perhaps a correction/determination of reference values for cows in the first lactation could be taken into consideration.

Summary

30 German Holstein cows were fed different amounts of minerals and vitamins in their first lactation. Group 1 received calcium, magnesium, phosphorus, copper, zinc, β -carotene and the Vitamins A and E as recommended (GfE, 2001) and Group 2 obtained at least double the amount of all components over the whole lactation. The concentrations of these elements were measured in the blood serum of all cows six times over a period of 37 weeks and compared with the present reference values. Concentrations below the minimum reference values in both groups and over the whole period were found for copper and β -carotene and for zinc only in Group 1. Significantly higher concentrations in the blood serum of Group 2 were found only for zinc and Vitamin E. Other differences between the animals of the groups (DMI, NEL, LW, FCM) or clinical deficiency syndromes were not detected.

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How does sample concentration affect measurement of antioxidant capacity?

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Introduction

For measuring antioxidant capacity of foodstuffs as well as of human plasma, numerous *in vitro* test systems exist. However, investigations about influences on the determination of antioxidant capacity are scarce. Several studies have shown a concentration-dependent antioxidant activity of ascorbic acid in peroxy radical trapping tests (Wayner et al. 1986, Frei et al. 1989, Lönnrot et al. 1996). In contrast, Benzie et al. (1999) demonstrated that the antioxidant efficiency of ascorbate in the FRAP assay is not affected by its concentration. Miller and Rice-Evans (1997) also showed linear relations between different concentrations of ascorbic acid, uric acid, α -tocopherol and trolox and inhibition of absorbance in the TEAC assay.

Therefore, aim of the present study was to evaluate the effect of sample concentration on the antioxidant capacity of four single compounds (trolox, ascorbic acid, gallic acid and uric acid) as well as of three food extracts (strawberry nectar, white tea and tomato extract).

Materials and Methods

Stock solutions of Trolox (2.5 mmol/L), ascorbic acid (5.7 mmol/L), gallic acid (0.6 mmol/L) and uric acid (6.1 mmol/L) were diluted according to the linear range of the standard calibration curve for the respective assay, resulting in four to seven different concentrations. The strawberry nectar, the white tea and the tomato extract were diluted in the same way, resulting in three to seven different concentrations depending on the test system. Analyses of the four substances and the three food extracts were done in triplicate on three and two different days, respectively, within several months. Antioxidant capacity was determined by using four different test systems: two versions of the TEAC assay (Miller et al. 1993, 1996) named TEAC I and TEAC II, the FRAP assay (Benzie and Strain 1996) and the PCL assay (Popov and Lewin 1999). The total phenolics content was measured using the method of Folin-Ciocalteu (Singleton and Rossi 1965). The content of vitamin C in the foodstuffs was

measured photometrically (Speitling et al. 1992) after extraction with *meta*-phosphoric acid.

Results and Discussion

In the first version of the TEAC assay and in the PCL assay the delay in radical generation as well as the ability of antioxidants to scavenge the radical (TEAC: ABTS^{•+}, PCL: O₂^{•-}) is analysed. The TEAC II assay only determines the ability to reduce the radical cation by using preformed radicals. The FRAP assay measures the reducing properties of antioxidants towards Fe³⁺. The total phenolics content is a sum parameter for reducing ingredients because the Folin-Ciocalteu (FC) reagent is not only specific to phenolic compounds. Thus, it can also be used as another method for determining antioxidant capacity. Generally, a good correlation between the method of Folin-Ciocalteu and other antioxidant activity assays is observed. Due to the differences between the test systems Schlesier et al. (2002) recommended the use of more than one method for assessing the antioxidant capacity.

Table 1: Antioxidant capacity and content of total phenolics with increasing sample concentration

Sample	TEAC I	TEAC II	FRAP	PCL	Total Phenolics
Trolox	constant	constant	conflicting*	constant	conflicting*
Ascorbic acid	conflicting*	constant	constant	constant	constant
Gallic acid	constant	conflicting*	conflicting*	increasing	constant
Uric acid	conflicting*	conflicting*	constant	conflicting*	conflicting*
Strawberry nectar	constant	constant	constant	increasing	decreasing
Tomato extract	constant	constant	constant	increasing	fluctuant
White tea	constant	constant	constant	increasing	decreasing

*repeated measurements resulted in conflicting effects of dilution

For ascorbic acid and trolox no dependence of antioxidant activity on sample concentration in most of the assays was observed (**Table 1**). As already mentioned, ascorbic acid showed decreasing antioxidant efficiency in peroxy radical trapping tests. Wayner et al. (1986) held an increased autoxidation of ascorbic acid with increasing concentration responsible for this phenomenon. In contrast, Benzie et al. (1999) suggested that methodological characteristics of peroxy radical trapping tests, combined with the instability of vitamin C, are mainly responsible for the appar-

ently concentration-related antioxidant efficiency values. Furthermore, the authors found out that the reducing efficiency of ascorbate, measured in the FRAP assay, is not affected by its concentration, which is in agreement with our results.

Gallic acid and uric acid mainly showed conflicting results between measurements on different days. Gallic acid possessed the strongest potential in all tests while the ranking order of the other substances varied. These findings confirm previous observations showing several single compounds reacting differently in diverse assays for measuring antioxidant capacity, which is due to the different reaction mechanisms (Schlesier et al. 2002). In the PCL assay gallic acid showed no linear relation between sample concentration and lagphase (**Figure 1**), resulting in increasing antioxidant activity with increasing concentration after consideration of dilution factors. The same context was found for all food extracts in the PCL assay. This can hardly be explained because commercially available test kits were used and the definite composition of reagents is not known. So the exact reaction mechanisms are partly unclear.

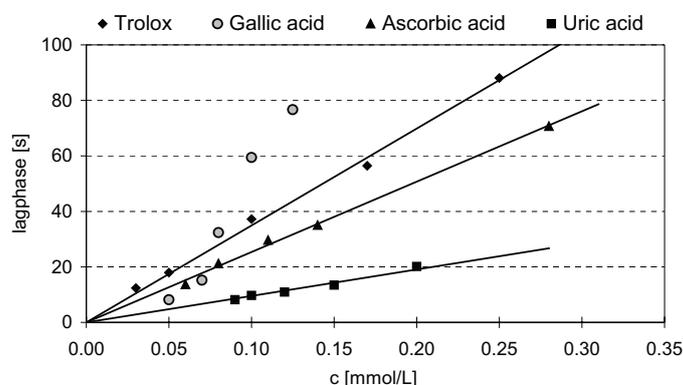


Figure 1: Relation between sample concentration and lagphase in the PCL assay

Within-run coefficients of variation for all assays and all single compounds tested were in an acceptable range (< 10%). Repeated measurements in longer time intervals resulted in considerably higher coefficients of variation which shows the problem of reproducibility. In contrast, repeated analyses of food extracts resulted in more reproducible effects of dilution (**Table 1**).

One food extract can behave contradictory in different tests. For example, the tea showed no effect of dilution on antioxidant capacity analysed by using the two versions of the TEAC assay as well as the FRAP assay, while in the PCL assay an increasing sample concentration resulted in higher antioxidant efficiency (**Figure 2**) and in the assay according to Folin-Ciocalteu in lower contents of total phenolics (**Figure 3**).

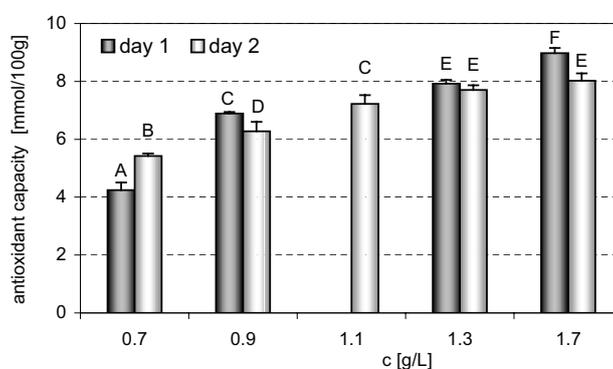


Figure 2: Antioxidant capacity of white tea in the PCL assay in dependence on sample concentration. Bars with same letters are not significantly different (ANOVA, $p > 0.05$)

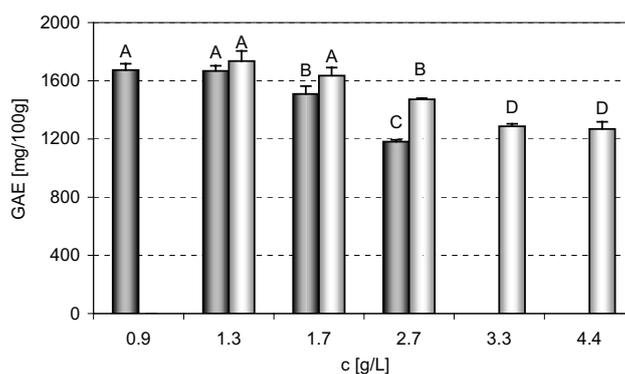


Figure 3: Content of total phenolics expressed as gallic acid equivalents (GAE) of white tea in dependence on sample concentration. Bars with same letters are not significantly different (ANOVA, $p > 0.05$)

On the other hand, several food extracts can show different effects of sample concentration in the same test. For tomato extract with low contents of total phenolics (**Table 2**), no definite effect of dilution was observed, but values fluctuated between different concentrations. For the remaining foodstuffs lower amounts of total phenolics with increasing sample concentrations were obtained. For the tea the effect of dilution seemed to be stronger than for the strawberry nectar. Thus, the strength of dilution effect in the Folin-Ciocalteu (FC) assay seems to depend on the amount of total phenolics in the sample. It must be noted that the FC reagent also reacts with ascorbic acid. Thus, the proportion of vitamin C on the “total phenolics content” of foodstuffs should be taken into account. The analysis of ascorbic acid in the FC assay gave a value of approximately 56 g GAE/100 g which was used to calculate the proportion of vitamin C on the total phenolics content of the food extracts (**Table 2**). The behaviour of the white tea seemed to be almost exclusively influenced by phenolic compounds other than ascorbic acid. Also in the strawberry nectar, the proportion of ascorbic acid on the total phenolics content was quite low (< 20%), while vitamin C amounted to 41-64% of the total phenolics content of tomato extract. This relatively high proportion of vitamin C may be an explanation for the constant total phenolic content over the whole concentration range because ascorbic acid was not affected by dilution in the FC assay.

Table 2: Proportion of vitamin C on total phenolics content (expressed as GAE) of the food extracts

parameter [mg/100g]	strawberry nectar [21 – 83 g/L]*	tomato extract [67 – 500 g/L]*	white tea [0.9 – 4.4 g/L]*
GAE	103 – 126	13 – 20	1181 – 1735
vitamin C	35 ± 1	15 ± 0	49 ± 4
GAE from vitamin C	20	8	28
percentage	16 – 19 %	41 – 64 %	1.6 – 2.3 %

* sample concentration used for analysis

Conclusions

For determining antioxidant efficiencies of food extracts the use of different versions of the TEAC assay as well as the FRAP assay is recommended. Measuring the photochemiluminescence using the Photochem[®] system is inadvisable at the

moment due to the influence of sample concentration on the results. Further investigations may elucidate this phenomenon. In general, it is suggested to choose a dilution around the middle of the standard calibration curve. For obtaining reliable results measurement of at least two different sample concentrations is advised. Further studies on the reproducibility of results in the different assays are required.

Summary

Aim of the present study was to evaluate the effect of sample concentration on the antioxidant capacity of four single compounds and three food extracts by using five different methods. While the single substances showed partially conflicting results for repeated measurements the food extracts provided more reproducible effects of dilution. The use of the two versions of the TEAC assay as well as the FRAP assay are recommended for determination of antioxidant capacity of foodstuffs. In the PCL assay gallic acid and all food extracts showed increasing antioxidant efficiencies when sample concentration increased. For strawberry nectar and the tea extract lower contents of total phenolics with increasing sample concentration was ascertained. For the tomato extract no effect of dilution on total phenolics content was observed.

Abbreviations

FRAP: ferric reducing antioxidant power, TEAC: trolox equivalent antioxidant capacity, PCL: photochemiluminescence

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Changes of the vitamin C concentration in the serum and of the tissue Total Antioxidant Status (TAS) in the precancerous states of the stomach cancer

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Introduction

In the pathogenesis of the diseases of the alimentary system the role of the reactive oxygenic species (ROS), which may directly damage the cells of the mucosa is more and more often recognized. The pathological states lead to an overproduction of ROS which develop structural changes of the enzymes, proteins, carbohydrates and of the fatty acids contained in the cells. The oxygenic radicals may cause a damage of the lipoproteins of the cellular membranes through the peroxidation of the lipids. Such changes are favoured by the failure of the antioxidant system (Bloch and Gartner, 1999; Hansonn, 1999; Giacosa and Filiberti, 1996; Montgomery et al., 1999). Vitamin C is considered as the most essential antioxidant of the extracellular fluid, important too for the antioxidant protection inside the cell. However, the antioxidant activity of the ascorbates is however questioned especially in their low concentrations. Then the ascorbates may act simply as peroxidants stimulating the reactions linked with the presence of transitory metal ions (Inslar and Helm, 1999; Levine, 1986; Oberbeil, 1997; Tolbert, 1982). Yet controversies are aroused as to the degree at which the deficiency of this system is associated with the development of the pathology of the gastric mucosa.

The aim of the study was to evaluate changes of the vitamin C concentration in the serum against the background of the total antioxidant status (TAS) of the gastric mucosa in considering the occurrence of the precancerous states of the stomach cancer.

Materials and Methods

The analysis included 91 patients (61 men and 30 women) aged 31 to 65 years who were examined because of pains in the upper part of the alimentary canal. In 31 of them chronic gastritis was diagnosed, in 23 – gastric peptic ulcer, and in the remaining 37 – duodenal ulcer. In the course of the diagnosis the following points were included:

1) gastroscopic examination with histopathological evaluation of the segments taken from the stomach stump mucosa;

2) the evaluation of the vitamin C concentration in the serum with the chromatographic method (HPLC). The supply of the vitamin C in all the persons analysed was standardized.

The range of the reference values of the vitamin C concentration in the serum amounting to 0.91 ± 0.18 mg/dl was established on the basis of the results obtained from the examination of 36 persons (21 men and 15 women), a group of blood donors aged 19 to 56 years.

3) the evaluation of the TAS of the gastric mucosa (Randox test, TAS reference values: 1.30 – 1.77 mmol/l).

In estimating the histopathological changes of the mucosa the following conditions were differentiated: normal mucosa, inflammatory changes, atrophic changes and dysplasia (Holleb et al., 1998; Urban, 1993).

Statistical analysis

The statistical analysis was carried out using a statistical program SAS for IBM PC rel. 6.03. A hypothesis of normal distribution was checked by the Shapiro-Wilk W test (SAS Procedure Guide rel. 6.03). The procedure UNIVARIATE was a program which was used to apply the above conditions. To study the statistical significance of differences between means (non-normal distribution) the Wilcoxon test was used. Data were deemed significant when $p < 0.05$.

Results

The vitamin C concentration in the patients examined varied from 0.22 to 1.29 mg/dl. The average vitamin C concentration amounted to 0.64 ± 0.29 mg/dl and was statistically significantly lower in comparison with the average reference value ($p < 0.001$).

The changes of the vitamin C concentration in the blood considering the intensification of the mucosal pathology are presented in table 1.

Table 1. Vitamin C concentration in the serum considering the occurrence of particular pathological changes of the gastric mucosa.

Histological changes of the gastric mucosa	Vitamin C concentration in serum Arithmetic mean average \pm Standard deviation [mg/dl]
Normal mucosa	0.84 \pm 0.16*
Inflammatory changes	0.63 \pm 0.19**
Atrophic changes	0.58 \pm 0.25 **
Dysplasia	0.51 \pm 0.18**

* to ** - statistically significant differences

From tables 1 it follows that the highest vitamin C concentration values in the serum were associated with the presence of the normal gastric mucosa. The appearance of any pathology on the ground of the gastric mucosa already statistically significantly lowers the average ascorbates supplies in the serum. However, the type of pathology forming on the ground of the gastric mucosa does not statistically significantly change the average vitamin C concentration.

The TAS of the gastric mucosa in the total number of patients examined varied from 0.31 to 1.96 mmol/l (average: 0.97 \pm 0.37mmol/l). The changes TAS in relation to the pathology of the gastric mucosa are presented in figure 1.

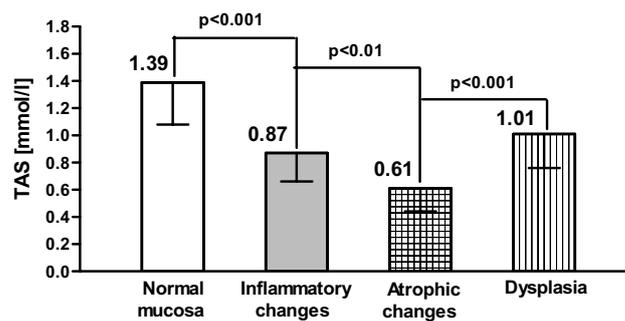


Figure 1. The TAS level in patients with various pathological changes of the gastric mucosa.

The results presented in figure 1 testify that the deepening pathological reconstruction of the gastric mucosa is associated with the statistically significantly decreasing TAS of the gastric mucosa. However, this tendency becomes disturbed in the dysplasia, in which cases the average TAS value statistically significantly increases. The lowest average TAS value amounting to 0.61 ± 0.17 mmol/l was associated with the atrophic changes of the gastric mucosa, on the other hand the highest, amounting to 1.39 ± 0.27 mmol/l occurred in the normal mucosa.

From the data presented in table 1 and figure 1 it follows that the changes of the vitamin C concentration in the serum are not fully synchronized with the decreasing TAS of the gastric mucosa. The decrease of the ascorbates supplies in the serum are indeed associated with the fall of the "mucosic" TAS, but this phenomenon only concerns the following sequences of the pathology:

changes of the normal mucosa → inflammatory changes → atrophic changes

Discussion

In the alimentary canal the main source of free oxygenic radicals are the mucoc-xanthic oxidase, to what McCord (1985) was the first to pay attention, and the NADPH oxidase contained in the neutrophil granulocytes. These cells can supply big quantities of a superoxide anion radical which through a series of reactions, may lead to the formation of one of the most active forms of oxygen, the hydroxylic radical (Bloch and Gartner, 1999; Stadtman and Berlett, 1997). The oxygen active forms may directly act cytotoxically on the gastric mucosa, lead to inflammatory changes, and to in further stages, to bowel metaplasia, stomach dysplasia, and cancer (Boyle, 1994; Gocosa and Filiberti, 1996; Hansonn, 1999). The enzymatic and non-enzymatic antioxidant mechanism protects the cells from the damaging action of the free radicals and our own studies were dedicated to the former. They indicated that as the pathology of the mucosa deepends starting from the normal mucosa, through the inflammatory changes, to the atrophic changes, the average vitamin C concentration in the serum as well as the average TAS value of the mucosa decreased what may suggest an increasing role of the ROS in the progression destruction of the gastric mucosa. A particularly clear, statistically significant fall of the ascorbates supplies in the serum occurred with the development of inflammatory

changes. This development of the pathology was associated also with the statistically significantly fall of the TAS. A further deepening of the deficit of "mucosic" antioxidants already remained without influence on the vitamin C supplies in the serum. Then the vitamin C in the serum, in the inflammatory and atrophic changes, may have reached a sufficiently low prooxidant concentration stimulating Fenton's reaction, since the increasing pathology of the mucosa was associated with a further fall of the TAS. The TAS decrease points to the intensification of the reaction of the ROS building up in the gastric mucosa. However the fact is surprising that with big ascorbates deficits in the serum, in the dysplastic changes the supplies of mucosic antioxidants are subject to reconstruction. This may also suggest the participation of other factors of destruction of the gastric mucosa marking their strongly impairing activity just at the stage of the developing of dysplasia.

We are convinced that the observations presented signal some problems, the exact understanding and explanation of which require further studies.

Conclusions

1. The increase of the pathology of the gastric mucosa, starting from the normal mucosa, through the inflammatory changes, to the atrophic changes is associated with the fall of the ascorbates concentration in the serum as well as with the decrease of the average TAS value of the gastric mucosa.
2. However, in the cells affected by the dysplastic changes, there comes to reconstruction of the supplies of the antioxidants despite a hitherto low vitamin C concentration in the serum.

Summary

The analysis included 91 patients (61 men and 30 women) aged 31 to 65 years were examined because of pains in the upper part of the alimentary canal. In the course of the diagnosis the following points were included: gastroscopic examination with histopathological evaluation of the segments taken from the stomach stump mucosa and estimation of the vitamin C concentration in the serum and of estimation the TAS of the latter. It was shown that the increase of the pathology of the gastric mucosa, starting from the normal mucosa, through the inflammatory changes to the atrophic

changes, was associated with the fall of the average ascorbates concentration in the serum and of the average TAS value of the gastric mucosa. However, in the cells affected by the dysplastic changes there comes to build up of antioxidants supplies despite a hitherto low vitamin C concentration in the serum.

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Importance of vitamin E supplementation during suckling and postweaning for α -tocopherol status and immune responses of piglets

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Introduction

Vitamin E is an important nutrient for the growth and health status of pigs, which are born with very low body fat reserves and low tissue vitamin E depots, factors that contribute to the sudden death of baby pigs. The low body store of fat-soluble vitamins is caused by a combination of low body store of lipids and restricted transfer of fat-soluble vitamins, such as vitamin E, across the placental barrier (Acuff et al., 1998). The second critical period for vitamin E faced by the baby pigs is during the first weeks after weaning probably due to the lack of enzymes required for the hydrolysis of the commercial source of vitamin E, all-rac- α -tocopheryl acetate, which is commonly added to weaner feed (Chung et al., 1992, Hedemann and Jensen, 1999, Lauridsen et al., 2001). Previous reports (Hidiroglou et al., 1993, Pehrson et al., 2001) have shown that supplementation of sow diets with vitamin E increases the vitamin E status of their progeny during suckling, but little is known with regard to the effect on the vitamin E depots of the progeny after weaning.

Vitamin C, a water-soluble vitamin, is not commonly added to pig feed as pigs are capable of synthesising vitamin C themselves. The interaction between vitamin E and C is interesting due to the reported sparing action on vitamin E or synergism between these two vitamins as shown in some model systems (Niki, 1987). Increased intake of vitamin C, therefore, may lead to improved vitamin E status.

Vitamins E and C have been found to increase the cellular and humoral immunity in pigs (Babinszky et al., 1991, Schwager and Schulze, 1998). A vitamin E deficiency has also been found to predispose pigs to different diseases, among them *Escherichia Coli* infection (Ellis and Vorhies, 1976). The effect of vitamin E on the immune system may be ascribed to modify the interactions of macrophages and lymphocytes (Afzal et al., 1984) or act as antioxidant on cells involved in immunological reactions is not well understood.

In addition to vitamin E and C, the effect of fatty acids on the immune system is highly recognized. In recent years, it has become clear that polyunsaturated long-chain fatty acids (PUFA) are important regulators of numerous cellular functions, including those related to inflammation and immunity. The key physiologic roles of PUFAs are as components of cell membranes and as precursors of eicosanoids

(Calder, 1997). PUFA of the n-3 family has an inhibitory effect on the proinflammatory cytokines, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and IL-8 produced by macrophages. These cytokines play an important role in the defence against infections.

The need for vitamin E supplementation when using fat with a high level of PUFA is generally accepted. In addition, the level of fat may influence the need for vitamin E, i.e. a minimum dietary level of 40 to 60 IU vitamin E/kg has been recommended with added fat, but 80 to 100 IU/kg may be necessary when diets are without supplemental fat (Moreira and Mahan, 2002).

The purpose of this project was to test different nutritional strategies to increase α -tocopherol and immune status of piglets. The first experiment involved the effect of increased dietary levels of vitamin E for lactation sows, and addition of vitamin C to the weaner feed. In the second experiment the effect of increasing dietary levels of vitamin E was tested in weaner feed containing 5% fat of varying fatty acid composition.

Materials and Methods

Experiment 1 involved 12 crossbreed sows that were fed increasing levels of all-rac- α -tocopheryl acetate (70, 150, and 250 IU/kg) during lactation. After weaning (28 d), piglets were fed the same amount of all-rac- α -tocopheryl acetate (70 IU/kg feed). In addition, piglets were fed either with addition of vitamin C (500 mg/feed) or without vitamin C.

Experiment 2 involved 7 litters of piglets obtained from sows fed 250 IU all-rac- α -tocopheryl acetate/kg feed, and the piglets were fed three dietary levels of vitamin E during the postweaning period (85, 150, and 300 IU all-rac- α -tocopheryl acetate/kg feed) from 28 to 56 d. The three dietary treatments were added to feed, which had been supplemented 5% of animal fat, sunflower oil or fish oil.

Results

Results of experiment 1 are detailed described by Lauridsen and Jensen (2005), whereas results of experiment 2 are unpublished. A summary of the results of both experiments are given here:

Experiment 1:

Experiment 1 showed that increasing maternal dietary vitamin E supplementation increased the concentration of α -tocopherol in plasma ($P=0.02$) and milk ($P=0.007$, Figure 1) of sows. When lipid-standardized, plasma α -toc was increased in suckling piglets of sows fed 250 IU of All-Ace compared with other sow-groups ($P=0.005$).

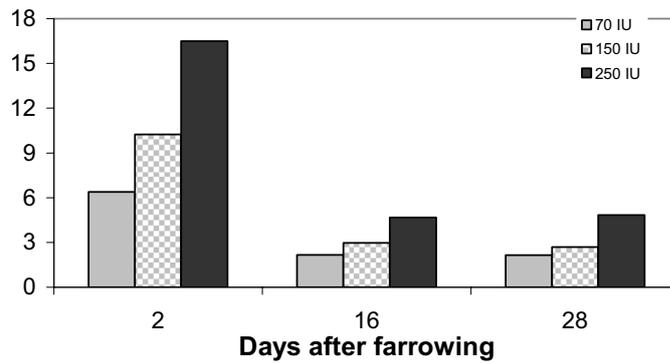


Figure 1: Effect of increasing dietary all-rac- α -tocopheryl acetate to sows on α -tocopherol (mg/L) in milk.

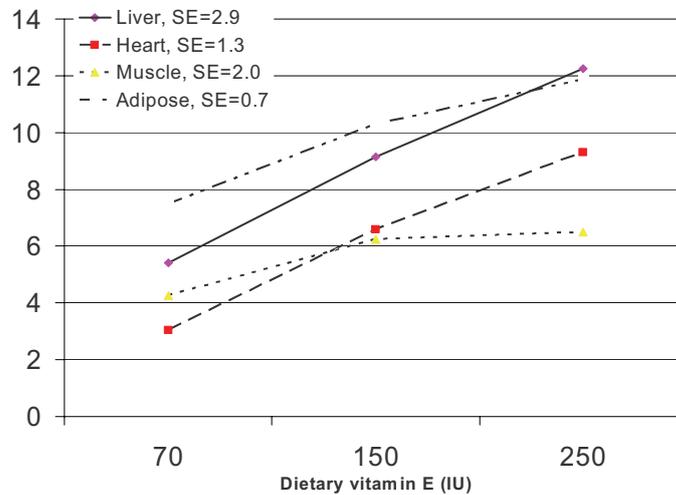


Figure 2: Effect of increasing dietary all-rac- α -tocopheryl acetate to sows on α -tocopherol (mg/kg) in tissues of piglets at weaning (day 28 of age).

At 28 d of age, α -tocopherol concentration in tissues was increased with supplementation of high all-rac- α -tocopheryl acetate levels to the sows (Figure 2); however, after weaning, a decrease in α -tocopherol concentration in most tissues (except liver) was observed (Figure 3). However, with regard to muscle and adipose tissue, tendencies for an interaction between age and sow treatment were observed, as the decrease in α -tocopherol concentration was less when 150 and 250 IU all-rac- α -tocopheryl acetate/kg was provided.

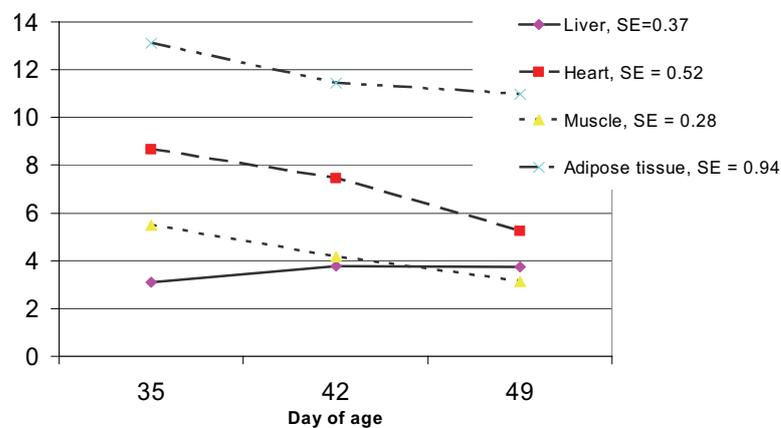


Figure 3: Effect of increasing dietary all-rac- α -tocopheryl acetate to sows on α -tocopherol (mg/kg) in tissues of piglets after weaning (weaned at day 28 of age).

Escherichia coli antibodies in serum of piglets increased with increasing age of piglets post weaning, and antibodies post weaning decreased with increasing dietary all-rac- α -tocopheryl acetate levels provided the sows. In addition, the number of litters treated against diarrhea post weaning was lower with higher levels of vitamin E provided to the sows.

The effect of vitamin C supplementation to the weaner feed was investigated on the α -tocopherol status and the humoral- and cell-mediated immune response. Addition of vitamin C to the weaner diet increased the concentration of α -tocopherol in the liver, and the concentration α -tocopherol in muscles was somewhat higher in piglets at day 35 and 49 of age, but lower at day 42 of age in vitamin C-supplemented piglets than in unsupplemented piglets.

Experiment 2:

Supplementation of 300 IU all-rac- α -tocopheryl acetate/kg feed to the piglets increased α -tocopherol concentration in serum at 42, 49, and 56 d of age compared

to 85 IU. However, no difference between the dietary treatments was observed within the first two weeks after weaning. At 56 d, α -tocopherol concentration of liver ($P<0.001$), adipose tissue ($P<0.001$), heart ($P=0.002$) and muscles ($P<0.001$) was increased in piglets fed 150 or 300 IU all-rac- α -tocopheryl acetate/kg feed when compared to 85 IU. Minor influence was observed with regard to the influence of dietary fat on the α -tocopherol concentration. When considering the difference between tissue α -tocopherol at 28 and 56 d, addition of 150 IU all-rac- α -tocopheryl acetate/kg feed seemed to be enough to maintain the vitamin E status postweaning in piglets weaned from sows fed 250 IU all-rac- α -tocopheryl acetate/kg feed. Minor influence was observed with regard to the influence of increasing vitamin E supplementation to piglets postweaning on their immune responses. However, supplementation of fish oil inhibited the production of PGE₂, LTB₄, TNF- α , and IL-8 by *in vitro* E. coli-lipopolysaccharide stimulated alveolar macrophages compared to addition of sunflower oil and animal fat.

Discussion and conclusion

Piglets suckling sows fed 150 or 250 IU/kg of all-rac- α -tocopheryl acetate had a higher α -tocopherol status at weaning (28 d of age) than did piglets suckling sows of 70 IU/kg. In sow milk, piglet plasma, and in some of the tissues, 250 IU of all-rac- α -tocopheryl acetate/kg increased α -tocopherol status compared with 150 IU/kg. Regardless of the dietary all-rac- α -tocopheryl acetate level for sows, addition of 70 IU of all-rac- α -tocopheryl acetate/kg to weaner diet was not enough to prevent a decrease in the α -tocopherol status in the tissues during the three weeks postweaning. However, supplementation of piglets with 150 IU all-rac- α -tocopheryl acetate/kg feed seemed to increase the serum vitamin E concentration in piglets from d 42 compared with supplementation of 85 IU all-rac- α -tocopheryl acetate/kg feed, and to be enough to maintain the tissue vitamin E status postweaning in piglets weaned from sows fed 250 IU all-rac- α -tocopheryl acetate/kg feed. Feeding of 150 IU/kg (or 300 IU/kg) was also enough to obtain a serum level of 1.5 mg/L, which is considered to produce a balance between serum and tissue (Moreira and Mahan, 2002). However, in order to supply the piglet with enough vitamin E to prevent the drop towards critical levels (below 1.5 mg/L) as frequently observed during the first two weeks postweaning, other nutritional strategies may be recommended e.g. vitamin C-supplementation or addition of vitamin E in a more absorption-facilitated way. In conclusion, nutritional strategies are available for lactating sows and their progeny, which can help in maintaining a proper vitamin E status for optimal growth and health status in pigs.

Summary

The purpose of this study was to test different nutritional strategies to increase α -tocopherol and immune status of piglets. The strategies involved dietary levels of all-rac- α -tocopheryl acetate (70, 150, and 250 IU/kg) in diets for lactating sows, and the effect of vitamin C supplementation postweaning, and in another trial the effect of increasing dietary levels of all-rac- α -tocopheryl acetate (85, 150, and 300 IU/kg feed) in diets supplemented with 5% of either animal fat, fish oil or sunflower oil was tested in diets for weaners. In conclusion, nutritional strategies are available for lactating sows and their progeny, which can help in maintaining a proper vitamin E status for optimal growth and health status in pigs.

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Effects of enhanced vitamin B supplementation in fattening pigs

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It is assumed that pigs with high lean tissue growth capacity require higher vitamin B amounts in the diet than the vitamin B concentration currently defined by the NRC (1998).

There is some evidence that high vitamin B supplemented diets improved lean meat percentage, immune status or fattening percentage in piglets and fattening pigs (Stahly and Cook 1996, Lutz and Stahly 1998, Coehlo 2001, Lutz et al. 1999 and 2002, Weiß and Quanz 2003). However, the reports have been more dealing with weanling piglets than with finishing fattening pigs (Cline and Hill 2002). The aim of the study was to obtain more information about the effect of higher vitamin B amounts on the performance of fattening pigs.

Material and Methods

Two groups of fattening pigs with different high vitamin B supplemented diets were on disposal of the investigation to clarify the effect of a dietary enhanced vitamin B content. One Group which is regarded as the control received a diet with a vitamin B supplementation like the diet used in the fattening performance test, the diet of the second group contained about eight times more vitamin B than the NRC standard (Table 1).

Table 1: Vitamin B concentration and supplementation of the diets of fattening pigs

Vitamins mg/kg diet	Vitamin B concentration in the diets without supplementation	Vitamin B supplementation of the diet	
		Control	High Vitamin B content
B1	4.3	-	+ 3.0
B2	5.0	+ 1.5	+ 15.5
B6	6.5	-	+ 2.0
B12	0.088	+ 0,010	+ 0.072
Panthontenic acid	11	+ 18	+ 94
Folic acid	0.60	+ 3.0	+ 56
Niacin	85	-	+ 2.0
Biotin	0.20	-	+ 0.050

In the Saxonia State Institute of Agriculture Köllitsch one experiment was carried out with 15 fattening pigs per group (Pi x DE/DL, 10 castrated male and 5 female) kept on split floor (experiment A). The animals received a pelleted feed via “Acema automates” in one period and came in with an age of 10 weeks at a mean body weight of 28.2 kg/piglet.

In the Thuringian State Institute of Agriculture Jena – Remderoda two experiments were carried out with 16 castrated male fattening pigs (PIC, PiHa x C23) per group (experiments B and C). The pigs were kept in single boxes on concret floor with a few sawdust as litter from the age of 10 weeks. The fattening period was carried out in two parts, the prefattening period from a mean body weight of 27.2 kg (experiment B) and 28.1 kg/piglet (experiment C), respectively up to 65 kg, and the finishing period from 65 kg to 118 kg body weight. The components of the diets are shown in table 2.

Table 2: Components of the mixed diets (%)

Components	Experiment A	Experiment B		Experiment C	
	Fattening period	Prefattening period	Finishing fattening period	Prefattening period	Finishing fattening period
Wheat	38	17	14	42	29
Barley	39.2	20	20	23	48
Triticale		20	20		
Wheat feed meal		3	12		
Wheat barn		12	10		
HP Soy bean meal		8	4		
Soy bean meal	18.3	8		19	14
Rape cake		6	6		
Peas			8	10	5
Oil L10	1.0	2.4	2.4	2.5	1.1
Premixe	0.5	0.5	0.5	0.5	0.5
Mineral mixture	3.0	2.8	2.8	3.2	2.5

The analysis of the nutrients in the feed for group 1 and 2 showed a relatively good confidence in all experiments (Table 3). The supplemented mineral mixture was identical in all three experiments and amounted 10.000 I.U. Vit. A, 1.500 I.U. Vit. D3, 80 mg Vitamin E, 2 mg Vit. K, 300 mg cholin chloride, 125 mg Fe, 100 mg Zn, 50 mg Mn, 15 mg Cu, 1 mg I, 0.6 mg Co, 0.4 mg Se, 100 mg BHT, 750 FYT 6-phytase per kg diet.

Table 3: Contents of nutrients in the mixed feed (g/kg diet)

Contents	Experiment A	Experiment B		Experiment C	
	Fattening period	Prefattening period	Finishing fattening period	Prefattening period	Finishing fattening period
Energy (MJ ME)	13.1	13.6	13.3	13.6	13.1
Crude Protein	192	193	160	178	164
Lysin	10.3	11.7	9.6	11.1	9.6
Methionin	3.2	3.2	2.6	3.4	2.5
Methionin/Cystin	7.5	7.0	6.0	6.6	5.6
Threonin	6.3	6.5	-	6.3	-
Crude fat	30	42	54	46	35
Crude fiber	47	52	44	40	48
Crude ash	50	48	45	50	40
Starch	382	370	396	416	430
sugar	54	42	40	36	29
Ca	8.7	7.1	7.1	7.2	5.8
P	5.1	5.0	5.1	5.3	4.7

The results were statistically assessed with program SPSS for Windows (version 6.01, SPSS, Inc.).

Results

The higher vitamin B concentration in the feed did not take a significant effect on the feed intake of fattening pigs (Table 4). However, there was a tendency of a light reducing in the feed intake in the vitamin B supplemented group.

Table 4: The effect of vitamin B supplementation on the feed intake in fattening pigs (kg / animal and day)

Experiment (n;n)	Control group		Vit. B supplemented group		p	% ¹⁾
	SD	mean	mean	SD		
A (15;15)	0.30	2.40	2.36	0.03	n.n.	98
B (16;16)	0.11	2.82	2.79	0.14	n.n.	99
C (16;16)	0.05	2.92	2.91	0.07	n.n.	100

1)control group = 100 %; Vit. B supplemented group = x %

The daily weight gain took place on a high level (Table 5). Hence, vitamin B supplemented fattening pigs gained with 860 g, 959 g and 991 g 104%, 102 % and 101%, respectively, life weight than the pigs in the control groups. The higher the daily weight gain the lower the differences between the control and the vitamin supplemented groups. However, the differences remained insignificant.

Table 5: The effect of vitamin B supplementation on the life weight gain in fattening pigs (g / animal and day)

Experiment (n;n)	Control group		Vit. B supplemented group		p	% ¹⁾
	SD	mean	mean	SD		
A (15;15)	86	828	860	110	n.n.	104
B (16;16)	69	945	959	64	n.n.	102
C (16;16)	64	980	991	98	n.n.	101

1)control group = 100 %; Vit. B supplemented group = x %

The feed efficiency for 1 kg weight gain was insignificantly lower in the vitamin B supplemented groups compared to the control animals (Table 6).

Table 6: The effect of vitamin B supplementation on the feed efficiency in fattening pigs (kg feed/)

Experiment (n;n)	Control group		Vit. B supplemented group		p	% ¹⁾
	SD	mean	mean	SD		
A (15;15)	0.24	2.90	2.74	0.12	n.n.	94
B (16;16)	0.18	2.98	2.91	0.24	n.n.	98
C (16;16)	0.19	2.97	2.93	0.32	n.n.	99

1)control group = 100 %; Vit. B supplemented group = x %

The fattening pigs with higher vitamin B feed contented needed 94%, 98%, and 99% of feed to gain 1 kg weight gain than the animals of the groups with low vitamin B feed content.

The vitamin B supplementation did not significantly alter the percentage of lean meat in the fattening pigs (Table 7). In experiment A the lean meat percentage was reduced by 2.1 %-points in the group with higher amounts of vitamin B in the diet. 55.2 % lean meat were obtained in the control group after 106 fattening days whereas 53.1 % were measured in supplemented group after 101 fattening days.

Table 7: The effect of vitamin B supplementation on the percentage of lean meat in fattening pigs (%)

Experiment (n;n)	Control group		Vit. B supplemented group		p	% ¹⁾
	SD	mean	mean	SD		
A (15;15)	2,6	55,2	53,1	3,3	n.n.	96
B (16;16)	2,1	55,0	56,2	2,7	n.n.	102
C (16;16)	1,7	54,0	54,7	3,2	n.n.	101

1) control group = 100 %; Vit. B supplemented group = x %

However, 1.2 %-points more were obtained by vitamin B supplementation in experiment B after 97 and 96 fattening days, respectively, but only 0.7 %-points more in experiment C after 91 fattening days. The back fat thickness was increased by 1,3 mm in experiment A, and reduced by 1.1 mm in experiment B and by 1.0 mm in experiment C.

No differences were observed between the control groups and supplemented groups regarding animal loss due to selection and emergency slaughtering. In experiment A there was no animal lost, in experiment B there was a loss of one animal in the control group and two in the supplemented group whereas in experiment C two animal lost happened in the control, and one in the supplemented group. The indication for selection was rectum prolapse in five animals. The reason for that has to be clarified.

Discussion

The supplementation of the diet of fattening pigs with vitamin B1, B2, B6, B12, pantothenic acid, folic acid, niacin and biotin by about 8 times more than the NRC standard took an insignificant effect on the fattening performance. On the average of the three experiments 1.0 % lower feed intake, 2.2 % increased life weight gain, and 3.1 % improved feed efficiency were observed.

The results are in accordance with findings of Weiß and Quanz (2003), who reported an increased life weight gain and a significantly improved feed efficiency by an increased vitamin B supplementation to the diet of fattening pigs.

Especially in pigs with a high capacity for proteinaceous growth the supplementation of B vitamins to the diet increased the life weight gain more than in pigs with a moderate-lean strain of pig (Stahly et al. 1995). When single B vitamins were increased in the diet like folic acid, niacin and vitamin B1 no improvement was detected regarding the fattening parameters (Lutz et al. 1999) whereas the vitamin B6 supplementation increased the life weight gain and reduced the feed efficiency in piglets from 10 to 26 kg body weight (Lutz and Stahly 1998). Furthermore, the supplementation of pantothenic acid of the diet of fattening pigs did neither improve life weight gain nor feed efficiency but increased the lean meat percentage and reduced the back fat thickness (Stahly and Lutz 2001, Autrey et al. 2002).

In experiment B and C the lean meat percentage tended to be increased in vitamin B supplemented groups than in the control groups. However, the lean meat percentage was decreased in experiment A. It is unlikely, that the reason for the different result is caused by the longer fattening period in experiment A, but it might be more likely that the different genotypes took effect on the result.

Furthermore, the effect of vitamin B supplementation on the fattening performance seems to be higher when the pigs are treated to moderate than to low stress (Coelho 2001).

Summary

Two groups of fattening pigs with different high vitamin B supplemented diets were on disposal of the investigation to clarify the effect of a dietary enhanced vitamin B content. Group 1 which is regarded as the control received a diet with a vitamin B supplementation like the diet used in the fattening performance test, the diet of group B contained about eight times more vitamin B than the NRC standard.

The higher vitamin B content of the diet took an insignificant effect on the fattening performance. On the average of the three experiments 1.0 % lower feed intake, 2.2 % increased life weight gain, and 3.1 % improved feed efficiency were observed. There was no effect on the mortality. However, the percentage of skeletal muscle was diminished by 2.1 %-points in experiments A whereas an increased percentage of skeletal muscle by 1.2 % and 0.7 %-points was observed in experiments B and C.

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Investigations on the biotin requirement of broiler chicken

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Introduction

Biotin is one of the essential water-soluble vitamins, which cannot be stored in the body in contrast to fat-soluble vitamins. Its main function is as co-enzyme of all carboxylation reactions in the metabolism. Therefore, deficiency can lead to poor growth, dermal lesions, and increased mortality. In ruminants, horses, and pigs considerable amounts of biotin are microbially produced in the rumen or the lower intestinal tract, but for poultry an adequate continuous alimentary supplementation is necessary to avoid deficiency. Former investigations (Roth-Maier and Paulicks 2002) indicated a remarkable reduction of feed intake and growth performance of broiler chicken, when biotin concentration of the feed was only 20 % below the recommendations given by the German Gesellschaft für Ernährungsphysiologie (GfE 2000). Therefore a further trial was conducted to confirm the small allowance between optimal and suboptimal biotin supply.

Materials and Methods

A basic diet was mixed consisting of corn (70 %), casein powder (15 %), soybean meal (10 %), soybean oil (1 %), and a mineral-vitamin premix (4 %). Only components with a biotin availability of 100 % (according to Frigg 1976, Blair and Misir 1989) were used. This diet contained 224 g protein and 13.4 MJ AME_N per kg and covered the nutrient, amino acid, mineral, trace element, and vitamin requirements of broiler chicken (GfE 2000) with the exception of biotin. 105 µg/kg, i.e. 70 % of the recommended content of 170 µg available biotin per kg dietary dry matter corresponding to 150 µg available biotin per kg diet were analyzed. The basic diet was supplemented with 0, 15, 30, 45, 75 or 195 µg biotin per kg resulting in a total of six dietary feed mixtures with biotin concentrations corresponding to 70, 80, 90, 100, 120, and 200 % of recommendations. The biotin supplementations were analytically confirmed with microbiological analyzation using *Lactobacillus plantarum* (ATCC 8014) after acid hydrolysis with H₂SO₄ (Ball 1994). The growth of *L. plantarum* was measured photospectrometrically at 550 nm after incubation for 18 hours at 37 °C.

The feed mixtures were fed for 5 weeks to 6 groups of 60 male chicken (ROSS) each, which were housed in groups of 10 in cages in a climated three-floored battery stable. Every week, birds were weighed individually and feed consumption was recorded per cage. After 5 weeks all birds were fasted for 3-4 hours. Two animals per cage with a mean body weight were killed by stunning, followed by decapitation. Blood was collected in heparinized tubes and centrifuged (at 4 °C; 5000 x g for 20 minutes) to obtain plasma. The liver was withdrawn completely, weighed, frozen immediately, and stored as the plasma at –80 °C until analysation.

The content of biotin in the liver was analyzed microbiologically after freeze drying as described for the feed. The concentration of biotin in blood plasma was analyzed microbiologically accordingly.

Results

Data of growth performance of the chicken are presented in Table 1. Performance of those broiler chicken, which received 80 % or less than the recommended biotin supply (treatment I, II) was markedly reduced compared with those receiving biotin in amounts meeting or exceeding recommendations (treatments IV, V, VI). Particularly chicken in treatment I with a dietary biotin reduction of 30 % had a rather low body

Table 1. Performance of broiler chicken with varying dietary biotin supplementation

Treatment		I	II	III	IV	V	VI	
Biotin in the diet (% of recommendation)		70	80	90	100	120	200	p<
Final body weight	g	1739 ^b ±101	1788 ^{ab} ±75	1874 ^{ab} ±66	1872 ^{ab} ±109	1905 ^a ±105	1900 ^a ±102	0.05
Feed intake	g/d	75.1 ±4.9	75.8 ±4.2	78.2 ±2.5	79.0 ±4.6	79.1 ±3.2	80.9 ±2.1	0.10
Feed efficiency (final week)	g feed/g weight gain	1.86 ±0.20	1.71 ±0.08	1.58 ±0.10	1.63 ±0.10	1.66 ±0.24	1.67 ±0.17	0.10

^{a,b} means without same superscripts differ significantly (SNK test)

weight (1739 g) and low daily weight gain (49.8 g/d), resulting from a reduced feed intake (75.1 g/d). During the final week of the experiment they consumed 1.86 kg feed per kg body weight gain. Adding 15 µg biotin per kg diet (treatment II = 80 % of recommendations) increased performance of broiler chicken slightly, but growth and feed intake were still 6 % lower and feed consumption for growth was still higher compared with animals receiving adequate diets. Biotin supplementations of more than 30 µg per kg feed (treatment III corresponding to 90 % of recommendations) were not able to improve growth performance furthermore. Average body weight was 1890 g with mean daily weight gains of 55.5 g. But daily feed consumption was tendentially higher when the biotin content of the feed was twice as high as recommended.

The concentrations of biotin in blood plasma and liver of the chicken are shown in Table 2. In blood plasma, biotin concentration reflected the concentration of biotin in the diet. With 0.07 µg biotin per 100 mg blood plasma for treatment I (105 µg biotin per kg diet) and 0.54 µg biotin per 100 mg blood plasma for treatment VI (300 µg biotin per kg diet) the increase was linear. The content of biotin in the liver was also affected by the dietary biotin supply and rose from 2.17 µg/g to 4.61 µg/g. The differences between treatments were significant. However, there was a linear increase between treatment I and treatment V. Between treatment V and treatment VI this increase was much smaller.

Table 2. Concentration of biotin in plasma and liver of broiler chicken with varying dietary biotin supplementation

Treatment	I	II	III	IV	V	VI	p<
Biotin in the diet (% of recommendation)	70	80	90	100	120	200	
Biotin in blood plasma µg/100 g	0.07 ^a ±0.02	0.09 ^{ab} ±0.02	0.14 ^{bc} ±0.04	0.17 ^c ±0.05	0.27 ^d ±0.05	0.54 ^e ±0.06	0.0001
Biotin in the liver µg/g	2.17 ^a ±0.24	2.57 ^b ±0.14	3.24 ^c ±0.16	3.80 ^d ±0.33	4.31 ^e ±0.14	4.61 ^f ±0.26	0.0001

^{a,b} means without same superscripts differ significantly (SNK test)

Discussion

The present results showed marked reductions of growth performance in broiler chicken, when dietary biotin concentration was 20 % or more below recommendations. Daily feed intake was 4 %, daily weight gains were 5 % lower compared with adequate supplied animals. The difference in feed-to-gain conversion amounted to 8 %. This confirms the results of former investigations, which showed similar reductions in growth performance, when biotin supply reached 80 % of the recommendations (Roth-Maier and Paulicks 2002). Compared with other water-soluble vitamins, this is a rather narrow margin, which needs to be carefully observed in composing diets for broiler chicken. A further aspect to be considered is the dietary biotin source. Biotin from several feedstuffs is not completely available for broiler chicken. Particularly in wheat and barley, cereals, which have a rather high biotin content of 100 to 150 µg/kg (Frigg 1984) biotin availability was measured to be 20 % or less (Frigg 1976, Blair and Misir, 1989). In contrast, corn with a biotin availability of 100 %, contains only 70 µg biotin per kg. Consequently, the same amount of corn provides more available biotin than wheat. But even when corn is combined with soybean meal, as in the present diet, dietary biotin content is too low for a high growth performance.

A further problem in providing adequate biotin supply is, that the data base on the bioavailability of biotin from feedstuffs is rather small. Many commonly used feedstuffs for broiler diets have not been examined yet and the data, which are available are rather old (Frigg 1976, 1984).

The diagnosis of alimentary biotin deficiency in practical broiler feeding is often done by obvious deficiency symptoms: biotin deficiency generally causes skin lesions or hock disorders and increases mortality. In the present experiment, none of these effects really became obvious. Hence, they cannot be used as indicators for biotin deficiency.

Also the concentration of biotin in blood plasma is perhaps no reliable parameter. In fact, Blair and Misir (1989) suggested to associate plasma and liver biotin concentrations in excess of 0.4 µg/100 ml and 3 µg/g, respectively, with an adequate biotin status of broiler chicken. In the present investigation, this limit for plasma was only exceeded in treatment VI (0.54 µg biotin/100 g) with a dietary biotin concentration of 300 µg/kg. According to the regression equation (Fig. 1), 240 µg available biotin per kg diet (= 160 % of recommendations) would be necessary. This

seems to be too high to recommend. In contrast, hepatic biotin concentration exceeded the suggested limit of 3 µg/g already with a dietary biotin supply of 130 µg/kg = 90 % of recommendation (Fig 2). This value is perhaps too low with regard to growth performance and might indicate the minimum requirement.

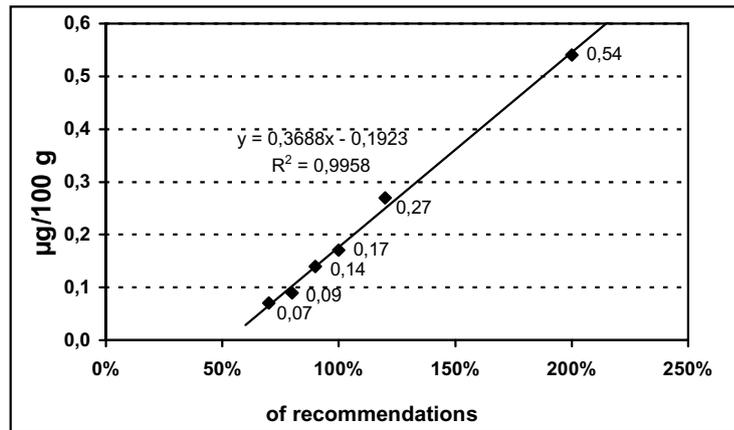


Figure 1. Biotin in blood plasma (µg/100 g)

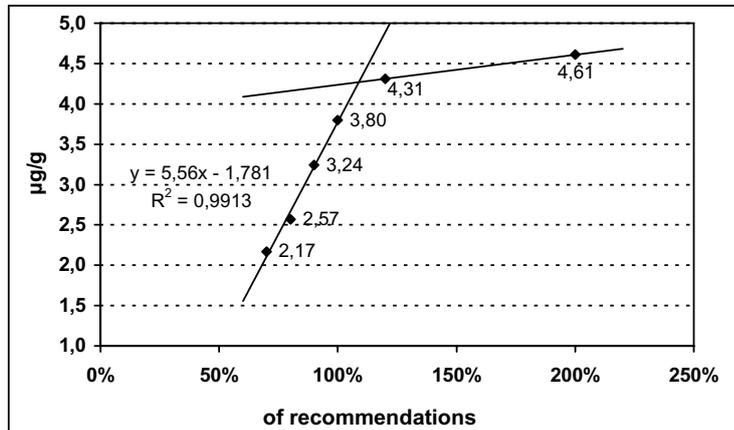


Figure 2. Biotin in the liver (µg/g)

In conclusion, the results of the present investigation confirm the actual German biotin recommendations for growing broiler chicken (GfE 2000). They also confirm former suppositions, that the allowance between optimal and suboptimal supply is very small. Together with a low biotin bioavailability from several commonly used feedstuffs as wheat, barley or cassava, feed mixtures for broiler chicken should generally be supplemented with biotin to avoid reductions in health and growth performance. As the data base on bioavailability of native biotin is small and therefore, also an exact extent is very uncertain, this supplementation should amount to the recommended dietary content of 150 µg/kg in diets based on wheat and/or barley, ignoring the native biotin concentration. In feed mixtures based on corn and soybean meal a biotin supplementation of 70 µg/kg diet might be sufficient.

Summary

360 male one day old broiler chicken (ROSS) divided into 6 treatment groups were fed for 5 weeks with feed mixtures containing 70, 80, 90, 100, 120, and 200 % of recommended content of available biotin, which amounts to 150 µg/kg diet. Feed intake, body weight gain, and feed-to-gain efficiency were markedly impaired by more than 6 %, when dietary biotin supply was 80 % or less of biotin recommendations. Biotin concentration in blood plasma reflected dietary biotin content linearly. Biotin content in the liver rose linearly up to 120 % of the dietary allowance, the further increase was reduced. Due to the small allowance between optimal and suboptimal biotin supply and the low biotin availability in several feedstuffs, a general biotin supplementation in broiler feeds is recommended.

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(1→3),(1→6)-beta-D-Glucan: Effects on blood parameters and performance of chickens

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Introduction

With current high intensity poultry husbandry, commonly at high stocking densities, flocks are extremely susceptible to stress and infectious agents. Even though the conditions in modern farms are reasonable well more often than not, chickens are caged or subjected to sorting, transport, vaccination and other perturbations. Particularly at risk are strains with a high capability for growth or egg-laying. That and/or a somewhat deteriorating hygienic environment may compromise the immune potential of poult and hens. In addition excess intake of micronutrients (e. g. vitamins A and E, PUFA), supplemented to increase immune responsiveness, or feed of poor quality can even have adverse effects on the antimicrobial defence (Sklan et al., 1997; Friedman et al., 1998). In the past antibiotics added to feed or water of farm poultry should thwart negative effects of pathogens on health, well-being, growth and performance. The EU policy to ban antibiotic supplementation to feed provoked severe efforts for substitute strategies. Immune-potentiating feed additives are such an approach. While antibiotics are targeted on the pathogen the immune-modulator strengthens the animals individual capability to withstand the challenge by micro-organisms or viruses. Immune-modulators in animal husbandry are not used as therapeutic agents, but they fulfil prophylactic objectives, e. g. in periods of infective risks. In most animals the immune system accounts for about three percent of the body mass and its constituents can be found all over the body. Its cells communicate among themselves and with others by various signal molecules in a more or less specific mode. In principle many chemical compounds from natural sources or synthesized chemically intervene with this network. Those which activate white blood cells are customarily termed immune-stimulants. However, cellular, local and systemic effects might be Janus-faced: the response to such a substance could be mediated by pro-inflammatory as well as anti-inflammatory cytokines. Therefore, the terms „biological response modifier“ or „immune-modulator“ are frequently used to take into consideration this problem.

Already in the 1940's the immune-activating and tumour growth inhibiting action of zymosan – a crude yeast cell wall extract – was noted. Later (1→3),(1→6)-beta-D-glucans were identified as the principally active compounds acting on several white blood cells, e. g. monocytes/macrophages, neutrophils and natural killer cells. These polyglucosides are widespread in fungi, bacteria, algae, and mushrooms and gained attraction owing to immune-modulating, anti-inflammatory, antimicrobial, antiviral, anti-tumour, radioprotective, and wound-healing properties. The biological activity depends on numerous structural requirements: molecular weight, triple-helix structure, length and frequency of branches, chemical modifications (Bohn & Miller, 1995; Mueller et al., 2000).

For many years, innate immunity has been considered to be of minor importance in anti-infective defence. In the last decade interest in innate immunity has grown tremendously (Janeway and Medzhitov, 2002; Uthaisangsook et al., 2002) and much work has been done to strengthen its anti-infective potential. The identification of

Dectin as the binding protein for (1→3),(1→6)-beta-D-glucan in phagocytes and its purification and biochemical characterization as pattern recognition receptor brought forward the elucidation of the cellular signal cascade triggered by this biological response modifier. The receptor is a small type-II membrane receptor (molecular mass 28,000) with an extracellular C-type lectin-like carbohydrate recognition domain fold and a cytoplasmic domain with an immunoreceptor tyrosine-based activation motif (Brown et al., 2001; Willment et al., 2001). This protein is expressed at high levels on macrophages and neutrophils, and to a lesser degree on dendritic cells and a sub-population of T cells (Taylor et al. 2002). The response to beta-glucan is dependent on the cooperation between Dectin-1, Toll-like receptor 2 and Myd88 (Brown et al. 2003). Toll-like receptors form functional pairs with other receptors and this interaction leads to phagosome assembling (Underhill et al., 1999) and cytokine production (Ozinsky et al., 2000). One important step in the intracellular signal cascade is the activation of the nuclear transcription factor kappa B, its transport into the nucleus, binding to DNA and propagation of cytokine synthesis (Battle et al., 1998; Hallman et al., 2001).

The macrophages and dendritic cells act as gateway between foreign pathogen invaders and the innate and adaptive immune system (Fig. 1). Peptidoglycans and lipopolysaccharides (endotoxin) of bacteria induce a proinflammatory response characterized by the synthesis of IL-1, IL-6, TNF- α , and acute phase proteins followed by fever, reduced appetite, and stagnating growth. In contrast beta-glucans initiate another route ensuing in high phagocytosis activity, antibody production, improved general performance of animals and enhanced resistance against high infectious pressure. The absence of microbial markers like beta-D-glucans or peptidoglycans etc. on the host cells comprises the basis of self *versus* nonself discrimination (Hoffmann et al., 1999). This property of phagocytes ensures instant response to foreign invaders and prevents attacking on own tissues.

The epithelial layer of gut is in permanent contact with bacteria. A layer of mucins and immune-globulin A prevents attachment and growth of bacteria, absorption of toxins and the inflammatory response to endotoxins and other harmful substances. Orally taken (1→3),(1→6)-beta-D-glucans interact with GALT – immune competent cells localized in Peyer's plaques and other agglomerations in the intestine. There dendritic cells and macrophages were activated and prime lymphocytes to force synthesis of IgA. A share of such lymphocytes migrates to bronchial, nasal, salivary, and mammary glands and to the skin, the urogenital tract and the oviduct (Withanage et al., 1999), where they secrete IgA. Scaling-up GALT by (1→3),(1→6)-beta-D-glucans is thus an approach to improve health and disease resistance of the whole body.

The basic biochemical and immunological studies of beta-glucan effects were made mainly on immune competent cells of mice and other standard laboratory animals. Experimental studies on whole animals including farmed animals (e. g. pigs, fishes) were frequently performed after microbial challenge. Feeding studies began with fishes, meanwhile the majority of studies were related with farmed pigs (Dritz et al., 1995; Decuyper et al., 1998; Engstad & Raa, 1999; Hiss & Sauerwein, 2003; Förster

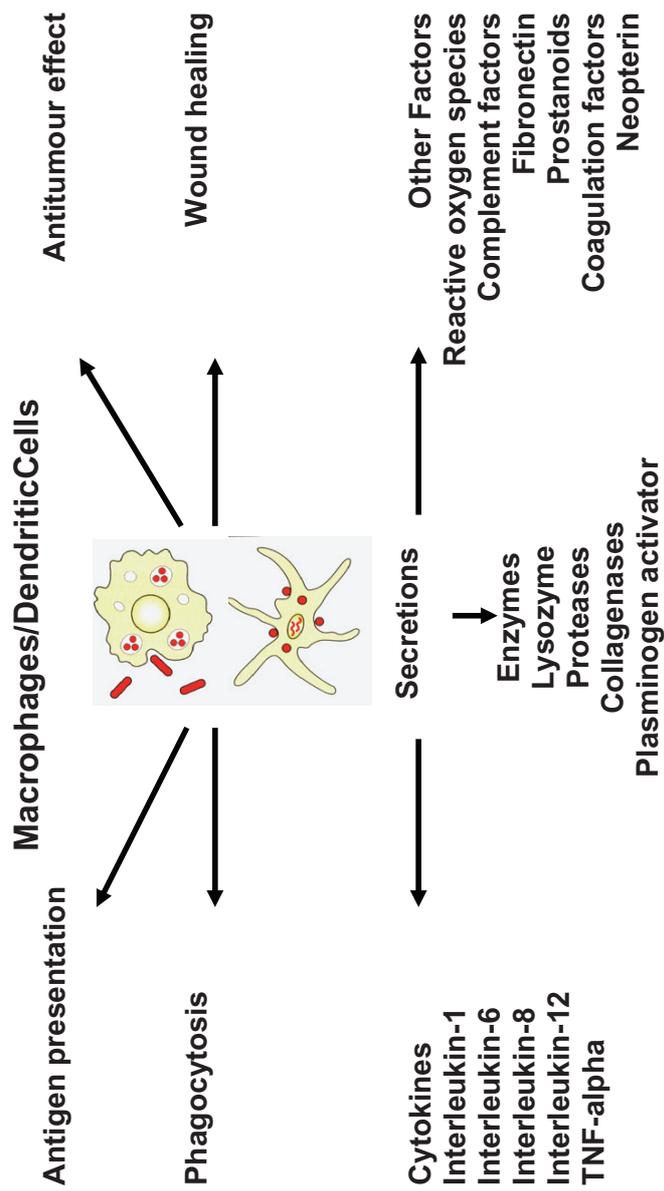


Figure 1. Diverse functions of macrophages and dendritic cell

et al., 2004; Förster et al., 2005. There are few reports on studies with poultry (Onifade 1997; Fleischer et al. 2000; Chen et al. 2003; Karaman et al. 2005; Lowry et al. 2005; Zhang et al. 2005).

This study is aimed to investigate the effect of (1→3),(1→6)-β-D-glucan of yeast on haematological and immunological parameters and some criteria of performance of laying hens and broiler chickens.

Methods

Broiler chickens: 200 chickens were allotted to two groups of equal size immediately after hatching. Leucogard[®], a cell wall fraction of *Saccharomyces cerevisiae*, was provided by Fibona Health Products GmbH Wiesbaden (Germany). The cereal feed of the intervention group was supplemented with 1.59 g Leucogard[®]/kg and given up to Day 15 of life. On Day 17 and Day 35 each half of the animals of the control group and the intervention group were killed by exsanguination.

Laying hens: The feed of 80,000 young laying fowls in an animal farm (living in two stables) was supplemented with 1.5 kg Leucogard[®] H per ton. 240,000 laying fowls (living in six stables at the same time) were taken as the control groups. Hens were housed in cages; five birds per cage. Three cages each of the control group and the intervention group were randomly chosen for blood analysis. The hens in these six cages were labelled. Blood was collected by aspiration from the wing veins 7, 14 and 47 days after start of feed supplementation with Leucogard[®] H.

Results and Discussion

In Table 1 the effects of Leucogard[®] on blood cells and plasma proteins in broiler chickens are summarized. Two main factors determine the changes in the parameters determined here: i.) the physical development of the poults, which may be seen in the control group on Day 17 and Day 35. ii.) the influence of β-glucan superimposing these developmental variations. Counts of erythrocytes and basophils and the concentration of haemoglobin are significantly different already on Day 17 between both groups with higher values in the trial group. Except the albumin concentration and the albumin/globulin ratio, which are lower in the trial group at this time, all the other values do not differ significantly between both groups (not shown here). From Day 17 to Day 35 there are highly significant increases of the following criteria in both groups: counts of leukocytes, heterophils, lymphocytes, and monocytes, and concentrations of haemoglobin, total protein, beta-globulin and gamma-globulin and decreases of alpha-globulin (not shown here). However, the increase of monocyte counts and the concentrations of total protein, beta-globulin and gamma-globulin and the decrease of heterophils, albumin/globulin ratio and albumin exceeds the changes in the control groups significantly. On Day 35 there are no differences in counts of erythrocytes and concentration between both groups.

From these it may be concluded: i. in the first five weeks of life chicken broilers the immune system matures as indicated by considerable changes in most blood cells and plasma proteins. These changes are modified by β-glucan in such a way that the share of lymphocytes and monocytes within the cell population and concentrations of gamma-globulin and beta-globulin within the protein fractions increase and the counts of heterophils and albumin and the albumin/globulin ratio decreases. These changes may be interpreted as an anti-inflammatory action of β-glucan.

Table 1. Effects of Leucogard® on blood cells and plasma proteins in broiler chickens

	Control	Trial	p
Erythrocytes (Tpt/l)*	2.63 ± 0.05 (34)	2.81 ± 0.09 (31)	<0.05
Haemoglobin (g/l)*	101.6 ± 2.8 (34)	110.7 ± 4.6 (31)	<0.05
White blood cells (Gpt/l)	27.7 ± 1.1 (43)	25.8 ± 1.5 (48)	ns
Heterophils (Gpt/l)	7.5 ± 0.4 (43)	4.8 ± 0.3 (48)	<0.000 1
Lymphocytes (Gpt/l)	18.3 ± 1.0 (43)	19.0 ± 1.4 (48)	ns
Monocytes (Gpt/l)	1.26 ± 0.15 (43)	1.54 ± 0.3 (48)	<0.05
Basophils (Gpt/l)*	0.10 ± 0.03 (34)	0.53 ± 0.07 (31)	<0.000 1
Total Protein (g/l)	30.35 ± 0.50 (43)	30.87 ± 0.6 (48)	<0.01
Albumin/Globulin Ratio	1.20 ± 0.04 (43)	0.96 ± 0.03 (48)	<0.000 1
Albumin (g/l)	16.30 ± 0.35 (43)	14.97 ± 0.25 (48)	0.002
Alpha-Globulin (g/l)	3.02 ± 0.12 (43)	3.10 ± 0.14 (48)	ns
Beta-Globulin (g/l)	5.24 ± 0.18 (43)	6.03 ± 0.15 (48)	<0.000 1
Gamma-Globulin (g/l)	5.78 ± 0.19 (43)	6.70 ± 0.22 (48)	<0.002

* represents values measured on Day 17

Table 2 represents the effect of Leucogard® feeding during 47 days on blood cells and plasma proteins in laying hens. The increase of white cell counts originates mainly from the accumulation of lymphocytes which surmounts quantitatively the decrease of heterophils. There are higher values in the trial group for basophils and the concentrations of total protein, albumin, alpha(2)-globulin, gamma-globulin as well as immunoglobulin G. The levels of alpha(1)-antitrypsin and haptoglobin tend to increase, however the differences are not significant.

These biological effects of β -glucan feeding are accompanied by improvements of egg-laying rate and mortality. During this feeding period layers in the control group (6 stables) produced 0.88 eggs/hen per day and in the intervention group 0.92 eggs/hen per day (2 stables), the rate of mortality within 60 days was 1.65 percent *versus* 1.08 percent, respectively.

The effects of β -glucan on heterophils, lymphocytes, gamma-globulin and IgG are in harmony with the conclusions on an anti-inflammatory mode of this immunomodulator in chickens.

There was another interesting observation on layers feeding β -glucan which is presented in the Figure 2. It shows the concentrations of bile acids in blood serum during the study. There took place a significant accumulation in the control group ($p = 0.003$), in the trial the change of bile acids was moderate and did not approach the level of significance. The level of bile acids in blood is an indicator for liver function. Kozhura et al.(1976) reported a decrease in the bile-secretory function of liver in white hens in the course of natural ageing.

Table 2. Effects of β -glucan on blood cells and plasma proteins in laying hens

	Control	Trial	p
White blood cells (Gpt/l)	35.7 \pm 1.6 (11)	44.0 \pm 3.9 (13)	0.06
Heterophils (Gpt/l)	20.7 \pm 1.8 (14)	15.6 \pm 1.3 (12)	<0.02
Lymphocytes (Gpt/l)	12.8 \pm 1.7 (12)	24.7 \pm 3.7 (13)	0.006
Basophils (Gpt/l)	0.61 \pm 0.16 (12)	1.03 \pm 0.18 (12)	0.09
Total Protein (g/l)	62.9 \pm 1.2 (14)	70.5 \pm 2.9 (15)	0.025
Albumin (g/l)	35.0 \pm 1.0 (15)	40.9 \pm 1.6 (15)	0.003
alpha(2)-Globulin (g/l)	3.6 \pm 0.2 (14)	4.5 \pm 0.3 (15)	0.02
Gamma-Globulin (g/l)	9.8 \pm 0.9 (14)	11.6 \pm 0.5 (14)	0.06
Immunoglobulin G	327.9 \pm 31.4 (14)	430.5 \pm 19.9 (13)	<0.01
alpha(1)-Antitrypsin	92.2 \pm 10.7 (11)	118.3 \pm 12.0(15)	0.1
Haptoglobin	154.8 \pm 17.5 (14)	191.7 \pm 17.2 (12)	0.15

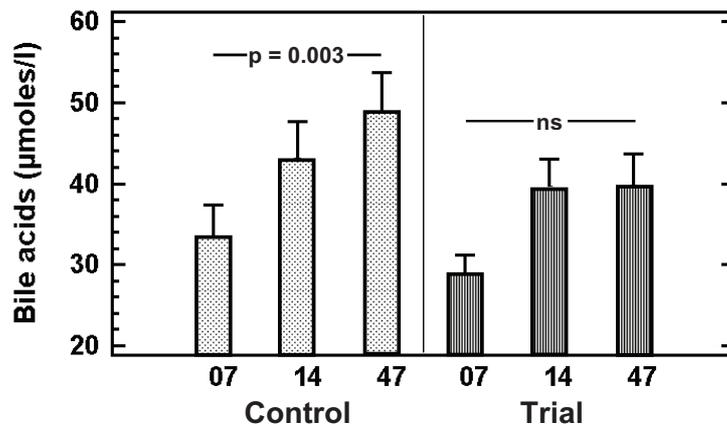


Figure 2. Influence of β -glucan on the accumulation of bile acids in blood of layers

Summary

A survey is given on the innate immune system and the effects on it. The macrophages and dendritic cells are of pivotal importance not only for phagocytosis, but also for coordination of the innate and adaptive immune response. (1 \rightarrow 3),(1 \rightarrow 6)- β -D- glucan interacts with pattern recognition receptors on these cells and initiate an anti-inflammatory response associated with increased anti-infective potential. This

may be concluded from decreased counts of heterophils and enhanced counts of lymphocytes and elevated concentrations of gamma-globulins in blood of broiler chickens and laying hens.

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Aspects of nutritional assessment of feeds from genetically modified plants (GMP) with output traits including beneficially acting substances

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Introduction

The cultivation of genetically modified plants (GMP) increased from 1.7 million to 81 million hectare from 1996 to 2004 (James 2004). Scientists and farmers, but also consumers, are increasingly asking for nutritional assessment, including safety aspects, of feeds and food from those plants. Substantial equivalence (SE) was created as a framework for the compositional assessment of feeds and food from GMP of the so-called first generation (without substantial changes of composition or without output traits) and is widely accepted. The concept of SE is based on the idea that an existing plant used as food or feed with a history of safe use and known feeding value can serve as a comparator, when assessing the safety and the feed value of a genetically modified plant (OECD 1993). Results of studies with feeds from these plants were recently summarized by Flachowsky et al. (2005).

Feeds with intended beneficial physiological properties such as amino acids, fatty acids, minerals, vitamins and other substances, which are called GMP with output traits or GMP of the second generation, may contribute to higher feed intake of animals and /or improved conversion of feed/nutrients into food of animal origin, associated with lower excretion of nitrogen, phosphorus and other nutrients. A nutritional assessment of these feeds is not adequate on the basis of compositional equivalence, other types of studies are necessary. The objective of this paper is therefore to present some considerations for experimental procedures to assess feeds from GMP with output traits, esp. including beneficially acting substances. More details are recently given by Flachowsky and Böhme (2005).

Studies to assess feeds from GMP with increased contents of beneficially acting substances

Feeds with intended beneficial physiological properties such as amino acids, fatty acids, minerals, vitamins and other substances may contribute to higher feed intake of animals and/or improved conversion of feed/nutrients into food of animal origin

and lower excretion of nitrogen, phosphorus and other nutrients. Most of these substances belong to the group of feed additives used in animal nutrition. Depending on the claim of changes as a consequence of the genetic modification, the experimental designs must be arranged to demonstrate these effects. Various experimental designs are necessary to show the efficiency of changes concerning nutrients or constituents:

- Bioavailability or conversion of nutrient precursors into nutrients (e.g. β -carotene)
- Digestibility/bioavailability of nutrients (e.g. amino acids, fatty acids, vitamins)
- Efficiency of substances, which may improve nutrient digestibility/availability (e.g. enzymes)
- Utilization of substances with surplus effects (e.g., prebiotics)
- Improvement of sensoric properties/palatability of feed (e.g. essential oils, aromas)

Apart from the intended increase of desirable substances, genetic modification has also proved to cause some side effects, as recently discussed by Cellini et al. (2004). Such secondary changes have to be considered, when GMPs of the second generation are assessed concerning their nutritional value or their safety. Animal studies which serve as the basis of comparative approaches are necessary to answer the questions mentioned. One of the most important questions concerning nutritional assessment of GMP of the second generation is the formulation or the type of controls. In many cases it can be presumed that the isogenic comparator is not available. Therefore a special experimental design must be created to assess the GMP of the second generation. Basic questions such as the optimal species or category of animals, their age, their keeping conditions and the extent and type of measurements have to be considered. More details for animal experimentation (e.g. number of animals, duration of experiments, composition of diets, measurements) are proposed by ILSI (2003, 2004).

Conversion of nutrient precursors

Balance studies with target animal species/categories are necessary to assess the conversion of nutrient precursors (e.g., β -carotene) into nutrients. At least two groups of animals are necessary for this purpose (Table 1).

Table 1: Proposal for assessing the conversion of nutrient precursors from GMP of the second generation into nutrients (e.g. β -carotene)

Group	Diet composition	Measurements
1 ¹	Balanced diets including typical levels of the isogenic counterpart + β -carotene (level/s adequate to the transgenic crop)	Depends on the claim of genetic modification: - concentration of converted substances in the target organ (e.g. vitamin A in liver) ²
2 ¹	Balanced diets with adequate amounts of transgenic crop	- metabolic parameters

¹ equal feed amounts for all animals

² until a steady state is achieved in the target organs

Dose-response studies (at least three dosages) with the supplemental precursor and the GMP of the second generation (adequate dosages) could improve the assessment, but are more expensive in terms of time, money and feeding material.

Assessment of various substances with beneficial effects (e.g. enhancer of nutrient utilization, substances with surplus effects or sensoric properties) in GMP

Expression of substances which improve nutrient utilization (e.g. enzymes like phytase or non starch polysaccharides degrading enzymes), which influence processes in the digestive tract (e.g. prebiotics such as oligosaccharides, fructans) or essential oils and other substances which improve sensoric properties or palatability is one of the objectives of genetic modification of plants.

The efficacy of these substances should be demonstrated using specific experimental designs (Table 2).

Table 2: Proposal to assess the effects of substances in GMP, usually used as feed additives (e.g. enzymes, prebiotics, essential oils etc.)

Group	Diet composition	Measurements
1	Balanced diet including typical levels of the isogenic counterpart, <i>ad libitum</i> feeding	Depends on the claim of genetic modification: - feed intake
2	Diet of Group 1 plus additive adequate to transgenic crop (or dose-response studies), feeding level of Group 1	- digestibility of nutrients - specific parameters (e.g. mineralization, microbial change in the digestive tract etc.)
3	Balanced diet including typical levels of the transgenic crop, feeding level of Groups 1 and 2	- animal's performances
4	Diet of Group 2, <i>ad libitum</i> feeding	- metabolic effects (immune response, etc.)
5	Diet of Group 3, <i>ad libitum</i> feeding	- quality of food of animal origin

Further developments

In the future, GMP of the second generation with more than one modified output trait will be available. Experimental designs have to consider these claims and results must demonstrate the intended changes. It is fact that more experimental groups seem to be necessary in such cases as demonstrated in Tables 1 and 2. Case by case studies should be carried out to show the bioavailability/effect of each changed nutrient or of each decreased content of undesirable substances. If isogenic controls are not available, traditional hybrids should be used as comparators supplemented with adequate nutrients (Table 3).

Table 3: Proposals for the nutritive assessment of feeds from GMPs of the second generation with more than one output trait

Group	Diet composition	Measurements
1	Balanced diet including typical levels of isogenic or near isogenic counterpart, <i>ad libitum</i> feeding	Depends on the claim of genetic modification: - analysis and <i>in vitro</i> measurements
2	Diet of Group 1 plus nutrients A, B..(adequate amounts of Diet 3), feeding adequate to Group 1	- availability/digestibility - indicator values
3	Balanced diet including typical levels of the transgenic crop, feeding adequate to Group 1	- feed intake - animal performances, feed efficacy
4	Diet of Group 2, <i>ad libitum</i> feeding	- incorporation in animal tissues
5	Diet of Group 3, <i>ad libitum</i> feeding	- quality of food of animal origin

Transgenic animals and fish might be available in the future. One example is the “phytase transgenic pig” as described by Golovan et al. (2001). The saliva of these pigs is intended to contain the enzyme phytase, which allows the pigs to digest phytate-phosphorus. Other enzyme excretions or metabolic processes may be also modified by genetic modification. But there is still a lot of research required to identify useful targets for genetic modification and to increase overall efficiency of the expensive genetic modification methods in food producing animals (Sang, 2003). Special studies are necessary to assess the modification of animals.

Summary

In the future, more feed from GMP will be available for animal nutrition as whole crops, crop components or co-products. Feeds from GMP of the so-called second generation (with output traits) are characterized by intended beneficial nutritive properties, such as increased contents of valuable nutrients or decreased concentrations of anti-nutritive substances. Specific animal feeding studies need to be conducted with the target species to confirm the expected nutritional properties of the modified crops, their components or co-products depending on the type of modification.

Some examples for adequate studies concerning nutritional assessment of such feeds have been presented as the basis for further discussions.

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Dense gas technology for innovative drug delivery systems

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Introduction

Modern therapeutically used drugs are mainly lipophilic compounds. To act on target structures, the drugs must be dissolved in physiological fluids and absorbed through entrance ports. In modern medicine oral application of solid forms is the preferential way. Since the bioavailability of orally applied drugs depends on the velocity of dissolution and absorption, methods to increase the dissolution rate are often necessary to reach significant blood levels. A well suitable way to increase the dissolution velocity is the reduction of particle size [1, 2]. Recent investigations show, that the Rapid Expansion of Supercritical Solutions (RESS) enables the formation of submicron particles of thermally labile drugs (Griseofulvin, Phytosterol, Ibuprofen). Depending on solvent and pre- and post-expansion conditions, particles can be produced with a mean diameter of 200 ± 50 nm [3-7]. Additional dissolution studies demonstrate that submicron Griseofulvin particles are characterized by a significantly higher dissolution rate [8]. However, submicron particles are difficult to be included in solid dosage forms. To overcome these problems, two different processes were developed: In the first process (Controlled Particle Deposition, CPD) the solid drug (Ibuprofen) must be soluble and the porous carrier (β -Cyclodextrin, β -CD) insoluble in a supercritical fluid. The key idea behind CPD is to dissolve the drug of interest in supercritical CO_2 , followed by permeation of the binary mixture into the pores of the carrier and precipitation of the drug inside the pores, caused by a fast pressure drop [9, 10]. Until now, much work was done on producing particles of pure solutes by RESS, but only a few studies investigated the simultaneous co-precipitation of two solutes [11-13]. Thus, the second process presented in this paper concentrates on particle formation using a modified RESS-process (CORESS) with a drug (Phytosterol) and a biodegradable polymer (Eudragit[®] or L-PLA) as solutes. For CORESS both, the drug and the polymer are dissolved in supercritical CO_2 , followed by the rapid expansion of the ternary mixture. This leads to the simultaneous co-precipitation of the solutes, resulting in the micro-encapsulation of the drug [7, 14].

Materials, Processes and Analysis

Ibuprofen 50 was supplied from Knoll Pharmaceuticals (UK), β -Cyclodextrin from Wacker-Chemie GmbH, Eudragit[®] from Röhm, and Carbon dioxide (CO₂) from Linde (Germany). Phytosterol was obtained from Fluka Chemie GmbH (Germany) and Poly(L-lactic acid) (L-PLA) was self-made. All other materials and solvents were of the purest grade available.

The CPD-apparatus enables experiments in the temperature range from 280 K to 350 K at pressures up to 50 MPa [9, 10]. All inclusion experiments are carried out using a static technique. Usually in these experiments, Ibuprofen and β -Cyclodextrin, are filled into separate cartridges inside a high-pressure cell ($V \approx 600 \text{ cm}^3$). For comparison, inclusion experiments with physical mixtures are performed, too. Thereby both, Ibuprofen and β -CD, are weighed out, thoroughly mixed, and packed into the cartridges. Then the high-pressure cell is sealed and immersed in a constant temperature water bath. Prior to inclusion experiments, the system is evacuated in order to remove atmospheric moisture and air. Thereafter, the required amount of liquid CO₂ is condensed into the high-pressure cell and heated to the desired temperature. As soon as pressure and temperature in the complex formation cell is reached, the mixture is stirred for 14 hours at constant conditions. At the end of the experiments, depressurization is performed as quickly as possible.

The CORESS-apparatus enables experiments in the temperature range from 300 K to 650 K and pressures up to 60 MPa [4]. In all experiments, the gaseous CO₂ is condensed, sub-cooled, and pressurized to the desired pressure with a diaphragm pump. To minimize the unsteadiness of the flow and to accelerate thermal equilibrium, pure CO₂ flows through the bypass section into the high-pressure vessel and is expanded through a capillary nozzle into the expansion chamber. After equilibrium, the bypass section is closed and the supercritical CO₂ flows through an extraction vessel, which is packed with the solute. In these experiments both, Phytosterol and the polymer, are thoroughly mixed and weighed out and then packed into an extraction vessel. The saturated supercritical solution flows through a tube into a high-pressure vessel where the pre-expansion temperature and pressure is adjusted. The supercritical solution is expanded through a capillary nozzle with an inner diameter of 50 μm and a length of 50 μm always down to atmospheric conditions [14].

A number of techniques were used for product characterization [7, 9, 10]. Both, the total amount and the free amount of Ibuprofen in the complex were determined by High Performance Liquid Chromatography (HPLC). Differential Scanning Calorimetry (DSC) was used for physical characterization of the original and the processed materials (melting point, heat of fusion, and crystallinity). The unprocessed and the processed substances were characterized by X-Ray Diffraction (XRD) and by Fourier Transform Infrared Spectrometry (FTIR). Dissolution studies were performed at 310 K at pH 5 using a dissolution model according to Stricker [15]. The size of the obtained particles was measured online with the Three-Wavelength-Extinction Measurement Technique (3-WEM) [4]. Scanning electron microscope (SEM) was used to observe the morphology of the particle surface.

Results: CPD-Process

The CPD-experiments were performed at 313 K at 25 MPa or 30 MPa. HPLC analysis was used to characterize the complex and the amount of Ibuprofen in β -CD was calculated according the following equation:

$$\% \text{ of inclusion} = \frac{(\text{total} - \text{free Ibuprofen content})}{\text{total Ibuprofen content}} \times 100$$

As shown in Fig. 1, the included Ibuprofen amount in the CPD-complex was found to be about 88 %. The experiments 1, 2 and 3 were performed at similar process conditions (circa 25 MPa, 313 K). Thereby, both Ibuprofen and β -CD were filled into separate cartridges. These results demonstrate the reliability of the experimental procedure and the complex characterization technique. In accordance with the results reported by Charoenchaitrakool et al. [16] no significant difference of the drug content was obtained as the static contact time (experiment 1, 2: 14 h; experiment 3: 6 h) was varied. In experiment 4 and 5 the two cartridges were filled with a physical mixture of Ibuprofen and β -CD for comparison. The included drug amount in the CPD-complex obtained from the processed physical mixture was found to be 60 % at 30 MPa and 55 % at 25 MPa, while the included amount of Ibuprofen in the unprocessed physical mixture (experiment 6) was only 2%. Dissolution studies using a dissolution model according to Stricker were performed at 310 K and at pH 5 for original Ibuprofen, its unprocessed physical mixture with β -CD, and a complex

formed by CPD. As shown in Table 1, both the dissolution rate coefficient (K_w) and the dissolved amount after 75 min. of the CPD complex was found to be significantly higher than that of original Ibuprofen and of its unprocessed physical mixture with β -CD [17].

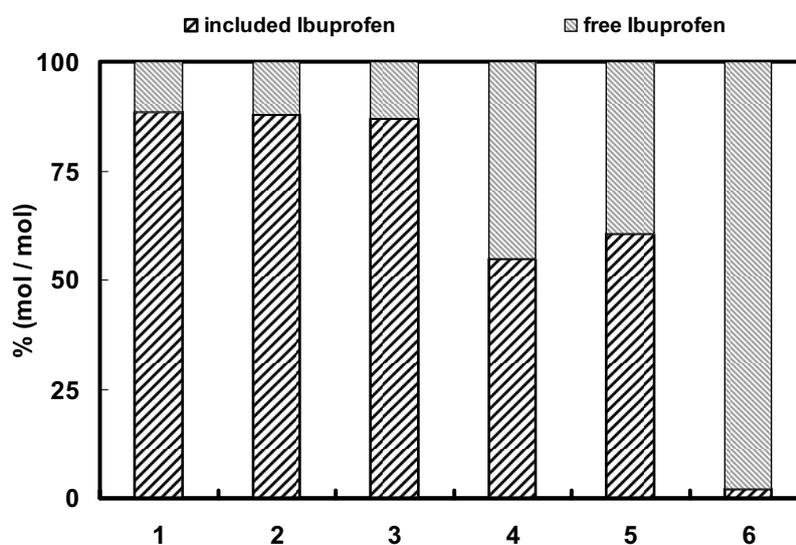


Figure 1: Amount of Ibuprofen in β -CD produced by different methods.

Table 1: Dissolution rate coefficients and dissolved amount of Ibuprofen.

	unprocessed Ibuprofen	unprocessed physical mixture	CPD processed physical mixture	CPD complex
K_w (min^{-1})	> 0.013	0.038	0.110	0.102
Dissolved amount	60 %	79 %	98 %	94 %

Results: CORESS-Process

All experiments were performed at pre-expansion temperatures between 348 K and 388 K and a pre-expansion pressure of 20 MPa [7, 14]. To avoid clogging, the nozzle temperature was equal or slightly higher (≈ 10 K) than the respective pre-expansion temperature. In Fig. 2 typical results of RESS-processed Phytosterol and of the coprecipitated Phytosterol / Eudragit[®] particles are shown. These SEM-pictures are

typical examples of the obtained product. The agglomerated Phytosterol particles consist of primary particles with a particle size between 50 nm and 150 nm and show a spongy structure with a high surface area. In opposite to pure Phytosterol the CORESS-experiments performed with a mixture of Phytosterol/Eudragit lead to finely dispersed particles in the range of ≈ 250 nm [14]. This result illustrates that the simultaneous co-precipitation of two solutes is a promising method to produce composite particles. These particles appear as a drug core encapsulated in a polymer coating. Similar results were obtained for Phytosterol and L-PLA; again these experiments lead to dispersed particles with ≈ 250 nm in diameter. DSC was used for the physical characterization (melting point, heat of fusion, and crystallinity) of the original and the processed materials. The DSC curve of pure Phytosterol shows a melting peak in the range between 403 K and 415 K, while the curve of the submicron Phytosterol / Eudragit[®] particles shows a melting peak between 398 K and 413 K. In case of the Phytosterol / L-PLA particles a broad melting peak between 388 K and 411 K was observed. The decrease of the heat of fusion was used to estimate the amount of the polymer in the mixture. Depending on the initial mass ratio of the mixture in the extraction vessel, the amount of polymer varies in case of Eudragit[®] from ~ 8 to ~ 23 wt-% and in case of L-PLA from ~ 12 to ~ 43 wt-%.

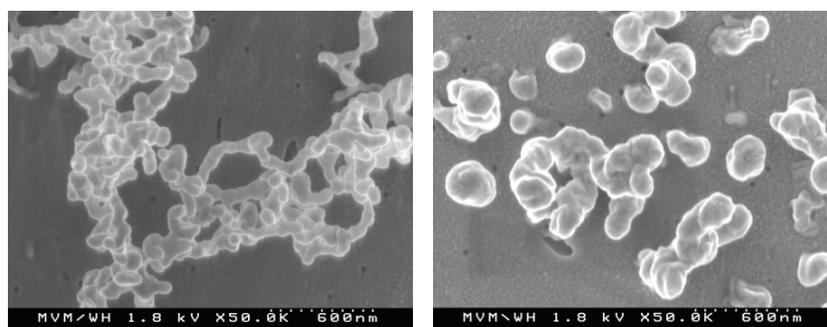


Figure 2: SEM of Phytosterol (left) and Phytosterol / Eudragit[®] (right) particles.

In addition, the original and the processed substances were characterized by XRD. In case of pure Phytosterol and of the Phytosterol / L-PLA mixture, diffraction peaks characteristic for crystalline Phytosterol are obtained. These diffraction peaks disappear for the submicron L-PLA particles. Thus, the L-PLA particles are in the

amorphous state. This result is in good agreement with the DSC analyses where no melting peak of crystalline L-PLA has been observed [7, 14].

Summary

The results of the present investigation can be summarized as follows: A successful inclusion Ibuprofen in β -CD has been achieved without using additional modifiers. The results demonstrate that the CPD process can be an efficient method for inclusion complex formation. The experiments with Phytosterol and biodegradable polymers (Eudragit[®] and L-PLA) lead to finely dispersed particles of \approx 250 nm in diameter. Thus, CORESS is a promising method for the formation of polymer-based drug delivery systems.

Acknowledgments

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***Trans*-11-18:1 is effectively Δ 9-desaturated compared with *trans*-12-18:1 in humans**

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Introduction

Trans vaccenic acid (*trans*-11-18:1, *tVA*) and *trans*-12-18:1 (*t12*) occur in ruminant fats as well as in partially hydrogenated vegetable oils (PHVO) in varying amounts. In ruminant fats, *tVA* is the predominant *trans* monoenoic (Precht *et al.* 2001) formed by the biohydrogenation of several PUFA (e.g., *cis*-9,*cis*-12-18:2) synthesised by rumen bacteria (Noble *et al.* 1974). As an intermediate mainly the *cis*-9,*trans*-11-CLA (*c9,t11*-CLA; Kepler *et al.* 1966; Griinari & Baumann, 1999) is formed but numerous other geometric and positional isomers of conjugated linoleic acids (CLA) are also created in this process. In cows the endogenous synthesis of *c9,t11*-CLA from *tVA* occurs mainly in the mammary gland and accounts for the main source of *c9,t11*-CLA in the milk and tissues (Griinari *et al.* 2000; Corl *et al.* 2001; Piperova *et al.* 2002). The endogenous desaturation of both *tVA* to *c9,t11*-CLA and *t12* to *c9,t12*-18:2 is catalysed by the stearoyl-CoA desaturase (E 1.14.99.5), also commonly known as Δ 9-desaturase (Griinari *et al.* 2000; Holman & Mahfouz, 1981; Pollard *et al.* 1980). Yet, the conversion of *t12* to *c9,t12*-18:2 in humans is still unknown. The potential health effects of CLA were reviewed in Belury (2002), Parodi (2004) and in Lee *et al.* (2005). At present, insufficient data is available concerning the isomeric distribution of *trans*-18:1 in different food sources and the human dietary intake of these individual isomers is generally unknown. The aim of the present human intervention study was to evaluate the endogenous Δ 9-desaturation of both *tVA* to *c9,t11*-CLA and *t12* to *c9,t12*-18:2 after a short-term (7 d) and a long-term (42 d) supplementation period. The CR of both *trans*-18:1 isomers were estimated by lipid analysis of serum and red blood cell membranes (RBCM).

Subjects and methods

The volunteers were selected after confirming that they were healthy, had a body mass index (BMI) >18 kg/m² and <30 kg/m². The age of volunteers was ranged 20 to

28 years (24 ± 3 y) and the BMI was between 19 kg/m^2 and 26 kg/m^2 ($21 \pm 2 \text{ kg/m}^2$). The subjects were normocholesterolemic ($4.4 \pm 0.7 \text{ mmol/L}$), had a LDL-C/HDL-C ratio < 3 and the triacylglycerol (TG) concentration was $1.0 \pm 0.4 \text{ mmol/L}$.

The study consisted of a 2-wk adaptation period and a 6-wk intervention period. During the complete study (8 wks) the volunteers had to consume a ruminant fat-free baseline diet. In the last wk of each of the study periods the volunteers consumed a standardised ruminant fat-free diet over the last 7 d. The diet of the *test*-group (*t*-group) was supplemented with 3.0 g/d of *tVA* and 3.0 g/d of *t12*. The *control*-group's (*c*-group's) diet was supplemented with a control oil to make the two treatment group's diets isocaloric. The *trans* FA mixture (*tFAM*) (Natural ASA, Norway) was comprised mainly of *tVA* and *t12* (1:1) and these two components constituted over 60 % of total FA in the preparation. The control oil was a mixture of palm kernel oil and rapeseed oil at a ratio of 1:1 without TFA and CLA. Both experimental fats (*tFAM* and control oil) were added to a commercial available chocolate spread. Blood samples were collected at 0 d, 7 d, and 42 d.

The lipid content of serum, RBCM and food samples were extracted according to Folch *et al.* (1957) and treated with NaOCH_3 (0.5 M NaOCH_3 in methanol, 15 min, at 60°C) to produce fatty acid methyl esters (FAME) extracts. The first GC method (C4 to C25 including total CLA) used a fused-silica capillary column DB-225ms (60 m x 0.25 mm i.d. with $0.25\text{-}\mu\text{m}$ film thickness; J&W, Scientific, USA). The second GC method separates the *cis* and *trans* isomers of 18:1 using a fused-silica capillary column CP-select (200 m x 0.25 mm i.d. with $0.25\text{-}\mu\text{m}$ film thickness; Varian, The Netherlands). The determination of the distribution of CLA-isomers was by silver-ion (Ag^+) HPLC (LC10A, Shimadzu, Japan). The exact details of the methodologies have been published in Kraft *et al.* (2003).

The estimation of the CR of *tVA* to *c9,t11*-CLA was calculated according to Turpeinen *et al.* (2002). The individual CR of serum *tVA* for each *t*-group subject was estimated by the net change of *c9,t11*-CLA ($\Delta c9,t11\text{-CLA}$) versus the sum of the net change of *tVA* (ΔtVA) and $\Delta c9,t11\text{-CLA}$ for each study period; 7 d compared 0 d to 7 d component levels (equation I) and 42 d compared 0 d to 42 d component levels, respectively.

$$[\text{I}] \text{CR} = \frac{c9,t11\text{-CLA}_{7d} - c9,t11\text{-CLA}_{0d}}{(tVA_{7d} - tVA_{0d}) + (c9,t11\text{-CLA}_{7d} - c9,t11\text{-CLA}_{0d})} \times 100\% \frac{Z_{7d-0d}}{E_{7d-0d} + Z_{7d-0d}}$$

Results

Serum lipids: The diet of *t*-group contained equal amounts of *t*VA and *t*12. The *t*-group's serum levels of *t*VA increased by 5- and 8-fold whereas serum levels of *t*12 increased by 9- and 12-fold after 7 d and 42 d of intervention, respectively compared with the adaptation period ($P \leq 0.002$). The *t*-group's serum *c*9,*t*11-CLA levels increased by the 1.7- and 2.0-fold after 7 d and 42 d of intervention, respectively compared with the adaptation period ($P \leq 0.001$). The concentration of *c*9,*t*12 18:2 remained unchanged in *t*-group's serum samples. In the *c*-group's serum levels of *t*VA, *t*12, and *c*9,*t*11-CLA remained relatively unchanged throughout the intervention and were significantly lower in comparison with the *t*-group ($P \leq 0.005$) (Tab. 1). The *t*-group's *t*VA CR for 7 d and 42 d was 24 ± 10 % and 25 ± 9 %, respectively. No conversion of *t*12 was determined. The CR of serum *t*VA ranged from 6 % to 40 %. Some subjects showed first a higher CR than that shown after 42 d of intervention or vice versa. One *t*-group subject indicated after 42 d the same content of *c*9,*t*11-CLA like in the adaptation period; no conversion was verified ("non-responder").

RBCM lipids: The *t*-group's RBCM *t*VA levels increased significantly by 5-fold ($P=0.002$) and *t*12 levels increased significantly by 9-fold ($P=0.002$) after 42 d when compared with the adaptation period (Tab. 1). In addition, the *t*-group's RBCM *c*9,*t*11-CLA levels increased significantly from 0.15 % to 0.18 % of total FAME ($P=0.021$) whereas no change of *c*9,*t*12-18:2 was observed (Tab. 1).

Table 1: The FA distribution of serum lipids and red blood cell membranes of the *test*-group and *control*-group during the study

FA (% of FAME)	ADAPTATION PERIOD		INTERVENTION PERIOD						
	Total subjects (n=24)		<i>t</i> -group (7 d) (n=11)		<i>t</i> -group (42 d) (n=11)		<i>c</i> -group (42 d) (n=12)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
	Lipids of serum								
<i>t</i> 11-18:1	0.07 ^a	0.02	0.35 ^b	0.09	0.52 ^c	0.10	0.07 ^a	0.02	
<i>t</i> 12-18 : 1	0.07 ^a	0.02	0.63 ^b	0.16	0.84 ^c	0.15	0.08 ^a	0.02	
<i>c</i> 9, <i>t</i> 11-CLA	0.16 ^a	0.04	0.27 ^b	0.10	0.32 ^b	0.10	0.15 ^a	0.06	
<i>c</i> 9, <i>t</i> 12-18:2	0.01	0.01	0.03	0.02	0.01	0.01	0.01	0.01	
	Lipids of red blood cell membranes								
<i>t</i> 11-18:1	0.09 ^a	0.01	- ¹	-	0.43 ^b	0.06	0.08 ^a	0.02	
<i>t</i> 12-18:1	0.10 ^a	0.02	-	-	0.87 ^b	0.15	0.11 ^a	0.04	
<i>c</i> 9, <i>t</i> 12-18:2	0.07	0.03	-	-	0.06	0.02	0.07	0.01	
<i>c</i> 9, <i>t</i> 11-CLA	0.15 ^a	0.04	-	-	0.18 ^b	0.05	0.08 ^c	0.02	

^{a,b,c} mean values within a row with different superscript letters were significantly different ($P < 0.05$).

¹Lipids of red blood cell membranes were analysed only 42 d of intervention.

In one *t*-group subject RBCM levels of *c9,t11*-CLA decreased by approximately half of its adaptation period levels over the 42 d intervention despite a large increase in the RBCM *tVA* levels. In addition, his ΔtVA was about 30 % higher than those of the other *t*-group *test* subjects. The so-called non-responder was excluded from the mean calculation (n=11) from lipid analysis of serum and RBCM of the *t*-group. After the 42 d period the *c*-group showed a significant diminishment of *c9,t11*-CLA by ~50 % compared with the adaptation period ($P \leq 0.01$), whereas *t12* and *c9,t12-18:2* were unchanged. The mean 42 d RBCM *c9,t11*-CLA (mean *c*-group; *c*) of *c*-group was used as the zero baseline to estimate the incorporation *c9,t11*-CLA into RBCM for individual subjects of *t*-group, see equation I.

$$[II] CR = \frac{(Z_{42d-0d}) + \Delta c9,t11-CLA(\text{mean } c)}{E_{42d-0d} + [(Z_{42d-0d}) + \Delta c9,t11-CLA(\text{mean } c)]} \times 100\% .$$

The calculated CR of *tVA* to *c9,t11*-CLA of *t*-group resulted in $19 \pm 3\%$ (equation II).

Discussion

The present study demonstrates that dietary *tVA* was effectively $\Delta 9$ -desaturated when compared with *t12*. Increased *tVA* concentrations in serum as well as in RBCM were associated with an increase of *c9,t11*-CLA concentrations in serum and RBCM, respectively (Tab. 1). The $\Delta 9$ -desaturase, an enzyme that desaturates SFA to MUFA (e.g., stearic to oleic acid), of rat liver microsomes converted the *tVA* to *c9,t11*-CLA and *t12* to *c9,t12-18:2* (Mahfouz *et al.* 1980; Pollard *et al.* 1980; Holman & Mahfouz, 1981). Previous studies in animals and humans observed the conversion of *tVA* to CLA (Ip *et al.* 1999; Kraft 2004; Banni *et al.* 2001; Santora *et al.* 2000; Loor *et al.* 2002; Gläser *et al.* 2000; Salminen *et al.* 1998; Adlof *et al.* 2000; Turpeinen *et al.* 2002). The results obtained support the endogenous synthesis of *c9,t11*-CLA from dietary *tVA* precursor in humans. Turpeinen *et al.* (2002) observed similar short-term results producing 307 % increase of serum *tVA* levels from a dietary intake of 3.0 g *tVA*/d (present study 400 %, Tab. 1). The increase of serum *tVA* was related with an increase of *c9,t11*-CLA in serum lipids in both this and Turpeinen's studies. The CR of serum *tVA* determined by Turpeinen *et al.* (2002) was on average 19 %. The CR obtained in this study are consistent with the results obtained after 42 d with 3.0 g *tVA* (CR 25 %). The calculation of the CR for *tVA* and *t12* is only a net end-product estimation. The ratio of changes of *tVA* and CLA and *t12* and *c9,t12-18:2*, respectively relative to the adaptation period do not reflect their real gross CR but the net sum of their surviving incorporated products. Kraft (2004) showed the highest

accumulation of endogenous synthesised CLA in tissues rich in neutral lipids, e.g., adipose tissue followed of gonads, thymus, kidney, muscle, liver, etc. However, the CR estimated in rats by changes of serum *tVA* and CLA was 22 % and represented the mean conversion of the total rat body (25 %; mean of all tissue CR) (Kraft, 2004). These results would suggest that the CR of *tVA* estimated by serum is as a good biomarker for the CR of the total human body.

In our study a 42 d intervention period was selected with the assumption that approximately one third of the RBC were renewed in that time. RBCM of pigs fed CLA (Stangl *et al.* 1999) and of rats fed the *tFAM* (Kraft, 2004) showed a linear increase of *c9,t11-CLA*, suggesting that RBCM are useful indicators for the FA incorporation in tissues. Obviously, *t*-group's dietary *tVA*, *t12* and endogenous synthesised *c9,t11-CLA* were incorporated in RBCM after 42 d (Tab. 1). In contrast, the content of *c9,t11-CLA* of *c*-group's RBCM was significantly decreased (Tab. 1). These results indicated clearly that the diet supplied to these test subjects was poor in TFA and CLA and that these subjects complied with the required study diet. Furthermore, in humans RBCM were assumed to be appropriate to the estimation of the CR of *tVA*, which was 19 ± 3 %. Altogether, the serum lipids are useful to estimate *tVA* CR whereas in long-term intervention the RBCM lipids are qualified to estimate FA incorporation and may produce improved *tVA* CR estimates.

The *t12* can be Δ^9 -desaturated to *c9,t12-18:2* (Mahfouz *et al.* 1980). Despite an increase of *t12* in lipids of serum and RBCM of *t*-group no increase of *c9,t12-18:2* concentration was observed (Tab. 1). Thus, no conversion of dietary *t12* could be assessed via serum and RBCM samples. In other studies, Salminen *et al.* (1998) analysed no individual *trans-18:1* isomers and Turpeinen *et al.* (2002) gave no detailed information concerning serum *t12* and *c9,t12-18:2* levels. In cows with abomasal infusion of a mixture of *tVA* and *t12* (1:1) these FA and their desaturation products *c9,t11-CLA* and *c9,t12-18:2* were incorporated into milk fat (Griinari *et al.* 2000). The increases of *t12* and *c9,t12-18:2* levels (64 %) were higher than the increases of *tVA* and *c9,t11-CLA* levels (40 %) whereas higher CR were observed for *tVA* (31 %) when compared with *t12* (10 %; Griinari *et al.* 2000). In rats fed *tVA* and *t12* (1:1) the CR of *tVA* was also higher than the conversion of *t12* (Kraft, 2004). In the present study, the mean increase of *t12* was generally 30 % higher than the mean increase of *tVA* including both the 22 % converted *tVA* in both serum and RBCM. The higher increase of *t12* levels is supposed in the literature and that in general *tVA* is preferentially metabolised when compared with *t12* by desaturation,

especially $\Delta 9$, elongation, and β -oxidation. Furthermore, Lippel *et al.* (1973) found that their CoA-esters had different rates of activation before desaturation.

The consumption of TFA and their effects on human health is still under review (EFSA 2004; Weggemans *et al.* 2004, Lock *et al.* 2005). The most important factor to consider when comparing *tVA* to other *trans*-18:1 isomers is that *tVA* is readily converted to the *c9,t11*-CLA. Several current studies demonstrated that the endogenous conversion of *tVA* via $\Delta 9$ -desaturase to *c9,t11*-CLA In animals this conversion was associated with anticarcinogenic effects (Corl *et al.* 2003; Miller *et al.* 2003; Lock *et al.* 2004). Further research is required into the mechanisms of *tVA* desaturation and the effects of individual *trans*-18:1 isomers on human health.

We can conclude from the present study, that *tVA* was effectively $\Delta 9$ -desaturated to *c9,t11*-CLA in humans. No whereas the conversion of *t12* to *c9,t12* C18 2 could not detected by serum and RBCM. After short-term intervention FA of serum could be used for the estimation of *tVA* CR with inter- and intra-individual variability. The mean CR of serum *tVA* was over the intervention period at 24 %. Under long-term conditions (42 d) the FA of RBCM were a more qualified biomarker for the estimation of *tVA* CR (19 %) due to the examination of incorporated FA.

The conversion of *tVA* to *c9,t11* CLA (¼) should be taken into account in future studies when determining the CLA supply.

Summary

***Trans*-11-18:1 is effectively $\Delta 9$ -desaturated compared with *trans*-12-18:1 in humans**

The aim was to evaluate the $\Delta 9$ -desaturation of *tVA* to *c9,t11*- CLA and of *t12*-18:1 to *c9,t12*-18:2 after a short-term (7 d) and a long-term (42 d) supplementation period. Serum *tVA* and *t12* levels increased after 7 d and 42 d ($P \leq 0.002$). The serum and RBCM *c9,t11*-CLA levels increased after 7 d and 42 d as well ($P \leq 0.001$) whereas in the control-group the RBCM *c9,t11*-CLA was decreased by 50 % ($P = 0.002$). The CR was calculated at 24 % by serum and 19 % by RBCM. No increase of *c9,t12*-18:2 was observed in serum and RBCM and thus, no CR of *t12* could be determined. In conclusion, the endogenous conversion of dietary *tVA* to *c9,t11*-CLA contributes approximately ¼ to the human body and should be considered when determining the CLA supply.

The list of cited literature can be request via E-mail: katrin.kuhnt@uni-jena.de

Accumulation of non-esterified sitosterol different in particle size in the tissues of the guinea pig

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Introduction

Phytosterols are used to lower the cholesterol concentration of the blood and for that purpose they are added into several nutrients. It was the intention to analyse whether there is an accumulation of sitosterol in the tissues of the guinea pig by sitosterol supplementation. Secondly the influence of two different particle sizes of sitosterol on sitosterol accumulation in different tissues was measured. In this context also the cholesterol concentration of the tested organs and tissues was verified.

Material and Methods

Two test substances were used: An original customary sitosterol product (O) consisting of 70% sitosterol, 16% sitostanol, and 14% campesterol having a particle size of 10 - 90 μm . The second substance tested was reduced to a submicron size of 0.2 - 0.3 μm (S) (Türk, 2004). To prevent agglutination of the particles an albuminous matrix was added. For the study 18 guinea pigs (Dunkin Hartley, female) were randomised into three groups with 6 animals each. Over two weeks they obtained either conventional feed (control) or sitosterol supplemented feed (O, S). The animals were killed on day 15 and test material was obtained (Fig. 1).

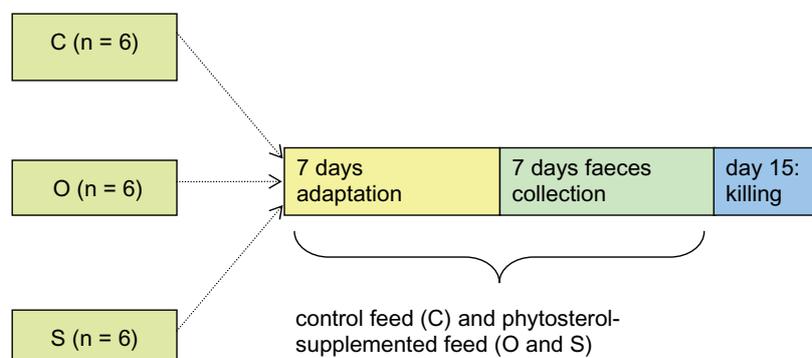


Figure 1: Study design

Ingesta, blood and tissues have been collected. Besides blood, the material was lyophilised, then hydrolysed (ethanolic NaOH, 1 h, 70 °C), extracted in cyclohexane, resolved in decane and the sterols were detected by GC-FID (Keller and Jahreis, 2004). Cholesterol and sitosterol were analysed in plasma, blood cells, tissues (liver, kidneys, gut segments), gut contents (jejunum, caecum, colon) and in faeces.

Results

The mean daily sitosterol intake was 21 ± 7 mg (control), 154 ± 8 mg (original), and 127 ± 18 mg (submicron), respectively. The sitosterol concentration of ingesta in different gut compartments and of faeces was significantly raised in both supplemented groups ($p < 0.05$). This is due to a very low absorption rate of phytosterols in general (Wester, 2000). The cholesterol concentration of the ingesta remained unchanged but was significantly raised in faeces of supplemented groups ($p < 0.05$) (Table 1).

Table 1: Sterol concentration in faeces [mg/g dry matter]

	Control	Submicron	Original
Cholesterol	0.26 ± 0.01^a	0.31 ± 0.02^b	0.32 ± 0.02^b
Sitosterol	0.53 ± 0.12^a	8.55 ± 1.69^b	8.26 ± 1.77^b

^{a,b} different superscripts mark significant differences ($p < 0.05$)

The cholesterol concentration in the analysed gut tissue was not significantly changed. In contrast, the sitosterol concentration was significantly increased in both supplemented groups ($p < 0.05$). The tissue of jejunum has been analysed separately as mucosa (containing the enterocytes) and serosa (Table 2).

Table 2: Sitosterol concentration of jejunal tissue [mg/g dry matter]

	Control	Submicron	Original
Mucosa	0.97 ± 0.37^a	2.82 ± 1.05^b	2.90 ± 1.36^b
Serosa	0.52 ± 0.20^a	1.17 ± 0.43^b	1.16 ± 0.59^b

^{a,b} different superscripts mark significant differences ($p < 0.05$)

The results show a higher sitosterol concentration in mucosa than in the appendant serosa. A possible mechanism of sitosterol absorption is, that the phytosterol is taken up into the enterocyte and is in a next step excreted back into ingesta via active transport mechanisms instead of being further transported into serosa and lymphatic system (Trautwein et al., 2003). Thus, only small amounts of sitosterol do actually attain the body tissues. The results of this study confirm this theory.

The cholesterol concentration of plasma and blood cells as well as of kidneys and liver was unaffected by the intervention. The sitosterol concentration of these matrices raised significantly ($p < 0.05$), there was only a tendency ($p < 0.1$) for the kidneys in the submicron group.

The intensity of the sitosterol accumulation is comparable in the single tissues (Table 3).

Table 3: Increase of the sitosterol level above the level of control [%]

	Serosa (jejunal)	Mucosa (jejunal)	Caecum-tissue	Colon-tissue	Plasma	Erythrocytes	Liver	Kidneys
Original	122	199	113	101	143	81	84	41
Submicron	123	191	119	95	82	87	105	32

Summary

Two different particle sizes of sitosterol were tested on its accumulation in different tissues of the guinea pig. The sterols of the collected tissues were measured.

The reduction of cholesterol was not significant in any of the tested tissues, also not in plasma. The insignificant but slight reduction of tissue cholesterol did not go along with an adequate sitosterol accumulation. There was a significant sitosterol accumulation independent of the particle sizes in all tested matrices which did not differ between the original phytosterol supplement and the submicron formulation. In order to judge the consequences of this accumulation, studies are needed to investigate the membranes and its stability, rigidity, fluidity and possibly also its permeability for various substances or particle sizes.

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Transfer of Antibiotics Used in Animal Husbandry from Slurry into Food

(Transfer von Antibiotika aus der Tierhaltung über Gülle in Nahrungsmittel)

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Low antibiotic concentrations are known to cause bacterial resistance in human and animal microbes. Little is known about the environmental effects of these drugs and their transfer into plants after using animal excreta as fertilizer. To achieve more information pigs were medicated twice with sulfadiazine (SD), trimethoprim (TMP) and chlortetracycline (CTC) and excretions collected. The slurry was administered as fertilizer on wheat and lettuce cultivations. Antibiotic residues in slurry, soil, wheat and lettuce were analysed by LC-MS/MS methods.

During 8 months storage overall antibiotic concentration of SFD decreased from 502 mg/kg slurry (1st medication period) and 559 mg/kg slurry (2nd period) to 29% and 50% of the original amount, respectively; CTC decreased from ~110 mg/kg slurry to 38% and 86 %. Overall administration of antibiotics to soil was 557 mg SFD and 176 mg CTC/m² after single and 922 mg SFD and 284 mg CTC/m² soil after double application of slurry. Antibiotic recovery rates from soil reached peaks after 2nd slurry administration and were reduced within 4 to 5 months to 32% and 17 % of maximum SFD and CTC recovery rates after single and 30% and 9% of SFD and CTC recovery after double slurry administration. Initially wheat roots contained 0.3 to 0.5mg SFD and 0.9 to 1.1mg CTC/kg DM. This amount was reduced to 0.1mg CTC/kg DM at harvest. Green leaves and stems contained 0.6 to 1.1mg CTC/kg DM but only traces of SFD. At harvest virtually no antibiotics were detected in straw, but wheat grain of the double fertilized plot 1 contained 0.05mg CTC/kg DM. Hence, strong evidence is given for the transfer of antibiotic residues in slurry via soil into plants which may be used for human consumption. Studies on several hydroponically grown plants reveal also the uptake of antibiotics by roots from nutrient and transport in plants.

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Effect of L-carnitine supplementation of sows on body composition and lipid metabolism of piglets

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Introduction

Recent studies demonstrated that L-carnitine supplementation of sows during pregnancy increases body weights of piglets and litters at birth (Musser et al. 1999; Eder et al. 2001; Ramanau et al. 2002). Furthermore, piglets of sows supplemented with L-carnitine showed a higher body weight gain during suckling period than piglets of control sows (Eder et al. 2001; Ramanau et al. 2002; Ramanau et al. 2004). Until now, the reasons for this increased pre- and postnatal growth of piglets are unknown, but possibly attributed to a different body composition at birth which leads to a higher growth capacity (Musser et al. 1999). Moreover there are observations in weaned piglets and growing pigs where L-carnitine supplementation leads to a lower content of lipids in the whole body as a result of increased rate of β -oxidation by risen activity of carnitine palmitoyltransferase I (CPT-1) (Owen et al. 1996; Heo et al. 2000; Owen et al. 2001a,b). Therefore, the aim of this study was to find out whether L-carnitine supplementation of sows could lead to alterations in body composition and lipid metabolism in their piglets at birth and during suckling period.

Materials and Methods

For this a total of 27 crossbred gilts (German land race x Large white) with an average body weight of 136 kg (± 9 , SD) were allotted to a treatment group (n=14) and a control group (n=13). All sows were inseminated with sperm of pietrain boars and fed commercially available diets with low carnitine concentrations (gestation diet: 10mg/kg; lactation diet: 3mg/kg). During pregnancy the sows received a basal diet ad libitum with a low content of energy (9.0 MJ ME/kg). The content of metabolisable energy sows received during lactation was 13.0 MJ ME/kg. Nutrient concentrations in the diets were (g/kg diet): pregnancy: CP (138), fibre (124), fat (27), ash (74); lactation: CP (173), fibre (45), fat (52) ash (52). On the day of farrowing the sows were fed 1.5 kg, which was successively increased (3 kg/d on d1 and d2 of lactation, 4.5 kg/d on d3 and d4 of lactation, ad libitum consumption from d5 of lactation to

weaning). Sows of the treatment group received per day additionally 125 mg of L-carnitine during pregnancy and 250 mg of L-carnitine during lactation as tablets containing L-carnitine (62.5 mg/tablet), lactose and dextrose (LAH, Cuxhaven, Germany). Five to six hours after birth, on d10 and d20 of lactation one piglet from each sow with a body weight representing the mean of the whole litter was selected and slaughtered to determine carcass composition (percentages of dry matter, crude protein, crude fat and crude ash; VDLUFA 1976). The piglets were euthanized by bleeding and blood samples were collected. Immediately after removal of the gastrointestinal tract caudal of the diaphragm and removal of liver, gallbladder and spleen the carcass was frozen at -20°C. The carcasses were first cut, then chopped and homogenized. A freeze-dried sample of 500 g was ground in a water-cooled grinder to a particle size of 1 mm. Chemical analysis was conducted on each sample in triplicate (VDLUFA 1976). In order to eliminate the effect of litter size on body composition and metabolism, the litter size of all sows was standardised to 8 piglets/litter within 2 days of farrowing. On days 80 and 100 of pregnancy sows were bled 6h after feeding by puncture of the vena cava. Plasma was obtained by centrifugation of the blood (4°C, 10 min, 3000U⁻¹). Lipids of the piglets' liver were extracted using a mixture of n-hexane and isopropanol (3:2, v/v) (Hara and Radin, 1978). Lipids of the extracts were dissolved in the aqueous phase of the test reagent with Triton X-100 (De Hoff et al. 1978). Concentrations of cholesterol and triglycerides were determined in piglets' plasma and liver lipid extracts using enzymatic reagent kits (Merck, Darmstadt, Germany). Five to eight hours after farrowing and on days 10 and 20 of lactation the sows were given 15 IU oxytocin by intramuscular injection. 50 ml milk was expressed manually from all active teats of each sow. The concentration of total carnitine in piglets' plasma and carcass, sows' plasma, milk and diets was determined by radiochemical method, which is based on the conversion of carnitine into [³H]acetylcarnitine by carnitine-O-acetyltransferase (McGarry and Foster, 1976). Means of the two groups of sows were compared by Student's *t*-test using a statistics package (Statistica for Windows, Version 6.0, StatSoft, Inc.).

Results and Discussion

In this study sows supplemented with L-carnitine had a higher feed intake during pregnancy (3.7 ± 0.4 vs. 3.3 ± 0.4 , $p < 0.05$) and a higher body weight on d 110 of

pregnancy (219 ± 12 vs. 210 ± 9 , $p < 0.05$) than control sows. Piglets and litters of sows supplemented with L-carnitine during pregnancy were 9% and 9%, respectively, heavier at birth than those of control sows (piglets: 1.40 ± 0.2 vs. 1.28 ± 0.4 kg/piglet, $p > 0.05$; litter: 14.4 ± 2 vs. 13.2 ± 2 kg/litter, $p > 0.05$). Although these differences were not statistically significant, it confirms that L-carnitine supplementation of sows has the potential to increase birth weights of litters and piglets (Musser et al. 1999; Eder et al. 2001a; Ramanau et al. 2002). The total number of piglets born and piglets born alive did not differ between control sows and sows supplemented with L-carnitine. But sows supplemented with L-carnitine showed a lower number of stillborn piglets than control sows (0.1 ± 0.4 vs. 0.8 ± 0.8 piglets, $p < 0.05$).

Table 1: Concentrations of total L-carnitine in plasma and milk of sows and in plasma and carcass of their piglets

	Control (n=13)	+ L-carnitine (n=14)
Plasma of sows ($\mu\text{mol/l}$)		
Day 80	8.0 ± 2.1	11.0 ± 3.8
Day 100	8.8 ± 0.8	14.9 ± 2.9^a
Milk of sows ($\mu\text{mol/l}$)		
Day 1 (colostrum)	183 ± 68	221 ± 45
Day 10	137 ± 28	212 ± 62^a
Day 20	126 ± 27	179 ± 41^a
Plasma of piglets ($\mu\text{mol/l}$)		
Day 1 (birth)	15.1 ± 2.2	20.0 ± 1.6^a
Day 10	15.6 ± 0.1	22.9 ± 2.2^a
Day 20	12.8 ± 0.7	17.5 ± 1.9^a
Carcass of piglets ($\mu\text{mol/g DM}$)		
Day 1 (birth)	0.94 ± 0.11	1.05 ± 0.14^a
Day 10	1.32 ± 0.15	1.73 ± 0.23^a
Day 20	1.39 ± 0.17	1.84 ± 0.28^a

^a Means are significantly different ($p < 0.05$); Data are Means \pm SD

Sows supplemented with L-carnitine had higher plasma concentrations of total L-carnitine on days 80 ($p < 0.15$) and 100 ($p < 0.05$) of pregnancy than control sows (Table 1). Piglets of sows supplemented with L-carnitine during pregnancy had also higher concentrations of total L-carnitine at birth in plasma and carcass. This shows that L-carnitine supplementation of sows improves the L-carnitine status of their piglets. This effect might be due to an increased transfer of L-carnitine from the maternal blood to the fetuses as a result of the higher plasma L-carnitine concentrations in mothers supplemented with L-carnitine. Milk of sows supplemented

with L-carnitine also had higher concentrations of total L-carnitine than milk of control sows at farrowing ($p < 0.10$) and on days 10 and 20 of lactation ($p < 0.05$). The higher concentrations of total L-carnitine in plasma and carcass of piglets of sows supplemented with L-carnitine at 10 and 20 days of age ($p < 0.05$) shows that suckling piglets of sows supplemented with L-carnitine have a better L-carnitine status than piglets of control sows. This might be due to the higher concentration of L-carnitine in milk of supplemented sows compared to milk of control sows. L-carnitine is very important immediately after birth because it is required for generation of energy by β -oxidation as the glucose supply is disrupted and glycogen stores are rapidly exhausted (Warshaw and Curry 1980).

Table 2: Composition of carcass of piglets of control sows and piglets of sows supplemented with L-carnitine at birth (day 1) and at days 10 and 20 of age

	Control (n=13)	+ L-carnitine (n=14)
Day 1 (birth)		
Body weight (kg)	1.32 \pm 0.26	1.38 \pm 0.26
Carcass weight (kg)	1.09 \pm 0.21	1.11 \pm 0.21
Dry matter (DM) (g/kg)	189 \pm 12	187 \pm 10
Crude protein (g/kg DM)	565 \pm 21	576 \pm 27
Crude fat (g/kg DM)	57 \pm 10	52 \pm 8
Crude ash (g/kg DM)	220 \pm 13	221 \pm 15
Day 10		
Body weight (kg)	3.35 \pm 0.45	3.41 \pm 0.50
Carcass weight (kg)	2.78 \pm 0.38	2.86 \pm 0.41
Dry matter (DM) (g/kg)	301 \pm 18	291 \pm 16
Crude protein (g/kg DM)	469 \pm 32	471 \pm 27
Crude fat (g/kg DM)	409 \pm 39	391 \pm 36
Crude ash (g/kg DM)	104 \pm 11	111 \pm 12
Day 20		
Body weight (kg)	6.58 \pm 0.82	6.55 \pm 0.68
Carcass weight (kg)	5.60 \pm 0.73	5.58 \pm 0.60
Dry matter (DM) (g/kg)	345 \pm 17	345 \pm 15
Crude protein (g/kg DM)	415 \pm 19	420 \pm 22
Crude fat (g/kg DM)	438 \pm 26	442 \pm 29
Crude ash (g/kg DM)	90 \pm 11	85 \pm 5

Concentrations of protein and ash in carcass dry matter decreased continuously from birth to day 20 while the concentration of fat increased continuously (Table 2). But the concentrations of these nutrients in the carcasses did not differ between piglets of control sows and those of L-carnitine supplemented sows at any time. This observation suggests that the improved L-carnitine status of piglets of supplemented

sows was not associated with alterations of the lipid metabolism, i.e. β -oxidation of fatty acids or synthesis of lipids. This finding disagrees with observations in weaned piglets and growing pigs where L-carnitine supplementation lowered the content of lipids in the whole body as a result of enhanced β -oxidation of fatty acids (Owen et al. 1996; Heo et al. 2000; Owen et al. 2001b).

Table 3: Concentrations of lipids in plasma and liver of piglets of control sows and piglets of sows supplemented with L-carnitine at birth (day 1) and at days 10 and 20 of age

	Control (n=13)	+ L-carnitine (n=14)
Total cholesterol, plasma (mmol/l)		
Day 1 (birth)	1.25 \pm 0.30	1.01 \pm 0.20 ^a
Day 10	3.06 \pm 0.38	3.11 \pm 0.71
Day 20	4.58 \pm 1.01	5.04 \pm 0.52
Triacylglycerols, plasma (mmol/l)		
Day 1 (birth)	0.42 \pm 0.26	0.46 \pm 0.26
Day 10	0.82 \pm 0.21	0.95 \pm 0.34
Day 20	0.67 \pm 0.12	0.88 \pm 0.33 ^a
Free fatty acids, plasma (mmol/l)		
Day 1 (birth)	0.22 \pm 0.10	0.23 \pm 0.12
Day 10	0.36 \pm 0.24	0.43 \pm 0.21
Day 20	0.27 \pm 0.10	0.32 \pm 0.16
Total cholesterol, liver (μmol/g)		
Day 1 (birth)	6.51 \pm 2.79	5.92 \pm 0.89
Day 10	6.14 \pm 1.51	5.01 \pm 1.97
Day 20	7.59 \pm 1.37	8.17 \pm 1.61
Triacylglycerols, liver (μmol/g)		
Day 1 (birth)	17.8 \pm 8.9	16.9 \pm 5.3
Day 10	8.3 \pm 3.8	9.7 \pm 2.5
Day 20	12.3 \pm 2.1	12.5 \pm 1.3

^a Means are significantly different ($P < 0.05$); Data are Means \pm SD

Piglets of sows supplemented with L-carnitine had lower plasma concentrations of total cholesterol at birth than piglets of control sows (Table 3). But the finding that the content of total lipid in carcass (Table 2), plasma triacylglycerols, plasma free fatty acids and hepatic concentrations of total cholesterol and triacylglycerols (Table 3) did not differ between neonatal piglets of sows supplemented with L-carnitine and those of control sows suggests that L-carnitine supplementation of sows influences neither lipid biosynthesis nor lipolysis in the fetus close to term. The only change in lipid parameters observed in suckling piglets of sows supplemented with L-carnitine compared with piglets of control sows was an increased concentration of

triacylglycerols in plasma on day 20 of age (Table 3). This could be due to an increased secretion of triacylglycerols from the liver into the blood or a reduced degradation of triacylglycerol-rich lipoproteins by lipoprotein lipase. But synthesis of triacylglycerols was probably not altered because the concentration of triacylglycerols in liver did not differ between both groups of piglets. The content of the other lipid parameters in plasma and liver did not differ between suckling piglets of sows supplemented with L-carnitine and those of control sows.

In conclusion, this study demonstrates that supplementation of sows improves the L-carnitine status of their piglets at birth and during the suckling period but does not influence their body composition or concentrations of lipids in liver, plasma and lipoproteins. This shows that piglets of sows supplemented with L-carnitine during the suckling period do not differ in their lipid metabolism from piglets of control sows. Probably the increased postnatal growth of piglets is not caused by a different body composition but is a result of a higher milk production of sows supplemented with L-carnitine which has been shown recently (Ramanau et al. 2004).

Summary

This study intended to investigate whether supplementation of sows with L-carnitine during pregnancy and lactation influences body composition and lipid metabolism of their piglets. A total of 27 crossbred gilts were allotted to a treatment (n=14) and a control group (n=13). Sows of the treatment group were supplemented with 125 mg L-carnitine/d during pregnancy and 250 mg L-carnitine/d during lactation. This study shows that supplementation of sows with L-carnitine improves the L-carnitine status of their piglets at birth and during the suckling period but does not influence their body composition and their lipid metabolism.

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Studies on the net absorption of L-carnitine in the ileum of broiler chicken

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Einleitung

L-Carnitin erfuh unter anderem wegen seiner hauptsächlich metabolischen Funktion, Fettsäuren durch die innere Mitochondrienmembran zum Ort der β -Oxidation zu transportieren, unter dem Aspekt der Energiebereitstellung für den Organismus in den letzten zwei Jahrzehnten zunehmendes Interesse in der Forschung.

Zahlreiche Experimente, die zum Einsatz von L-Carnitin beim Broiler durchgeführt wurden, zielten auf die Beeinflussung von Leistungsdaten, wobei die Ergebnisse wie in der Vergangenheit auch in den letzten 5 Jahren widersprüchlich waren (LIEN UND HORNG, 2001; BUYSE ET AL., 2001; RODEHUTSCORD ET AL., 2002; XU ET AL., 2003; CELIK UND ÖZTÜRKCAN, 2003; CELIK ET AL., 2003).

Grundsätzliche Fragen zum Absorptionsvermögen für L-Carnitin beim Geflügel wurden bislang nicht bearbeitet. In dieser Untersuchung sollten daher Fragen zur Höhe und Lokalisation der praecaecalen Nettoabsorption von supplementiertem L-Carnitin bearbeitet werden. Unbekannt war bisher auch, welchen Beitrag die Niere zur Exkretion von L-Carnitin beim Broiler leistet. Hierzu wurde in dieser Untersuchung eine Abschätzung vorgenommen.

Material und Methoden

Je 60 männliche Broiler (Ross) wurden mit einer von sechs Zulagestufen (0, 25, 50, 100, 200, 400 mg/kg) von L-Carnitin (Carniking®) zur Grundration (Tab. 1), die einem üblichen Broiler-Mischfutter entsprach (Energiegehalt: 14,9 MJ AME_N /kg T), versorgt.

Tab. 1: Zusammensetzung der Grundration

Futtermittel	[g/kg]	Futtermittel	[g/kg]
Mais	448	Dicalciumphosphat	20
Sojaextraktionsschrot (48 % XP)	283	Prämix	10
Weizen	100	Futterkalk	4
Weizenkleber	65	Viehsalz	3
Sojaöl	60	DL-Methionin	1
TiO ₂	5	L-Lysin-HCl	1

Die Grundration war bedarfsgerecht formuliert und beinhaltete 5 g TiO₂/kg als unverdaulichen Marker.

Die Käfige waren mit je 10 Tieren besetzt und ab dem 22. Lebenstag den Behandlungen zugeordnet (n = 6 Käfige je Behandlung). Nach 7-tägiger Fütterung und unblutiger Tötung aller Tiere mittels CO₂ wurde ein definierter Abschnitt des Dünndarms zwischen dem Meckel'schen Divertikulum und dem Ileum entnommen. Dieser Darmabschnitt wurde gedrittelt (mit proximal, medial und terminal bezeichnet), der Chymus je Drittel durch Spülen mit destilliertem Wasser gewonnen und für alle Tiere eines Käfigs gepoolt und gefriergetrocknet. Zudem wurden an den letzten drei Versuchstagen Stichproben der Exkremente gewonnen.

Die Bestimmung der Gehalte an freiem L-Carnitin (FC) und den drei kurzkettigen Estern Acetyl- (AC), Propionyl- (PC) und Hexanoyl (HC)-L-Carnitin erfolgte in allen Proben mittels ESI-MS/MS. Eine Methode dafür wurde in Zusammenarbeit mit dem Institut für Umwelt- und Lebensmittelchemie der Universität Halle-Wittenberg erarbeitet.

Die Berechnungen zur Nettoabsorption basierten unter Berücksichtigung der Marker- und L-Carnitin-Konzentrationen in Futter und Chymus (jeweils in g/kg T) auf folgender Gleichung:

$$\text{Nettoabsorption [\%]} = 100 - 100 * [(TiO_2 \text{ Futter} * L\text{-Carnitin}_{\text{Chymus}}) / (TiO_2 \text{ Chymus} * L\text{-Carnitin}_{\text{Futter}})]$$

Zur Schätzung der renalen Ausscheidung wurde die Differenz aus der Gesamtausscheidung und dem Fluss am terminalen Ileum herangezogen.

Alle ermittelten Daten wurden mit Hilfe des Programmes „Statistica“, Version 6.0 für Windows (StatSoft, Inc. 2004) ausgewertet. Zur Beschreibung der Ausscheidung und des praecaecalen Flusses wurde eine exponentielle Funktion an die Daten angepasst.

Ergebnisse und Diskussion

Nach Berechnung der Nettoabsorption für FC und einer zweifaktoriellen Varianzanalyse wurden keine Wechselwirkungen zwischen den beiden Hauptfaktoren Drittel des entnommenen Dünndarmabschnittes und Höhe der L-Carnitin-Konzentration im Futter festgestellt (Tab. 2).

Im Durchschnitt aller Dosierungen war die Nettoabsorption mit 84 % im terminalen Drittel signifikant höher als im proximalen Drittel des entnommenen Dünndarmabschnittes (79 %). Der signifikante Einfluss des Drittels auf das Absorptionsvermögen steht in Übereinstimmung mit Ergebnissen zur Nettoabsorption von Aminosäuren beim Broiler (KLUTH UND RODEHUTSCORD, 2004). Es wird geschlossen, dass am Meckel'schen Divertikulum die Absorptionsprozesse für FC nicht abgeschlossen sind. Deshalb wurden für weitere Berechnungen nur die Daten des terminalen Drittels verwendet.

Tab. 2: Nettoabsorption von L-Carnitin beim Broiler in den Dritteln des entnommenen Darmabschnittes nach unterschiedlicher L-Carnitin-Behandlung

L-Carnitin Konzentration [mg/kg]	entnommener Darmabschnitt [Drittel]	Anzahl Wdh. [n]	Nettoabsorption			
			MW [%]	s	MW [mg/d]	s
0		18	96 ^d	8	0,1 ^a	0,0
25		18	96 ^d	5	3,0 ^b	0,2
50		18	91 ^d	5	5,9 ^c	0,5
100		18	82 ^c	6	8,5 ^d	0,8
200		18	70 ^b	6	16,3 ^e	1,5
400		18	55 ^a	5	25,2 ^f	2,7
	proximal (p)	36	79 ^a	16	9,1 ^a	8,4
	medial (m)	36	82 ^{ab}	15	9,8 ^{ab}	8,5
	terminal (t)	36	84 ^b	16	9,9 ^b	8,4
0	p	6	98	5	0,1	0,0
0	m	6	95	11	0,1	0,0
0	t	6	96	8	0,1	0,0
25	p	6	93	8	2,9	0,3
25	m	6	96	4	3,0	0,2
25	t	6	97	3	3,1	0,1
50	p	6	88	7	5,8	0,6
50	m	6	90	4	5,9	0,4
50	t	6	94	4	6,1	0,4
100	p	6	75	5	7,8	0,7
100	m	6	84	3	8,8	0,6
100	t	6	86	3	9,0	0,7
200	p	6	64	5	14,9	1,1
200	m	6	71	3	16,4	1,0
200	t	6	76	2	17,6	0,9
400	p	6	55	5	25,4	2,8
400	m	6	56	5	25,5	3,2
400	t	6	54	7	24,7	2,7

n = Chymusproben gepoolt von 10 Tieren; ANOVA, zweidimensional; ^{a-d} in verschiedenen Spalten bedeuten signifikante Unterschiede nach Tukey's HSD Test mit $P \leq 0,05$

Ein signifikanter Einfluss der L-Carnitin-Behandlung war gegeben. Mit steigender Konzentration von L-Carnitin im Futter nahm die Nettoabsorption kontinuierlich ab, von 96 % in der Gruppe ohne L-Carnitin-Zusatz bis auf 55 % bei der höchsten Dosierung von 400 mg/kg. Diese 55 % entsprechen bei einer täglichen Aufnahme von 46 mg L-Carnitin immer noch 25 mg *netto* absorbiertem L-Carnitin je Tag und Tier. Mit jeder höheren L-Carnitin-Dosierung im Futter nahm die täglich absorbierte Menge von L-Carnitin signifikant zu (Tab. 2).

Einen quantitativen Vergleich der absoluten Mengen von freiem L-Carnitin im Chymus des terminalen Drittels (praecaecaler Fluss) mit denen in den Exkrementen (Gesamtausscheidung) ergab das in Abb. 1 gezeigte Bild.

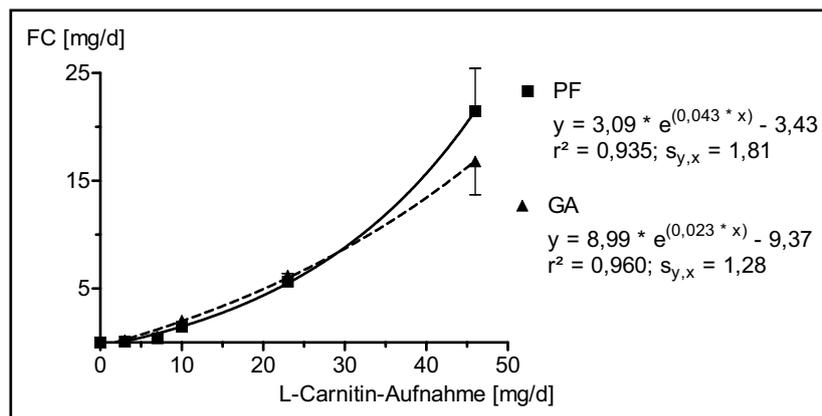


Abb. 1: Vergleich (Mw, s) zwischen praecaecalem Fluss (PF) und Gesamtausscheidung (GA) von FC beim Broiler nach unterschiedlich hoher L-Carnitin-Aufnahme

Bis zu einer täglichen Aufnahme von 23 mg FC/d, was einer Dosis von 200 mg/kg entspricht, war kein Unterschied der FC-Mengen in Chymus und Exkrementen zu erkennen. Bei einer Dosierung von 400 mg/kg wurden 4,7 mg FC mehr im Chymus als in den Exkrementen gefunden. Es wird deutlich, dass die Niere bei der Ausscheidung von FC beim Broiler im Gegensatz zum Säuger (BROOKS UND MCINTOSH, 1975, RUFF ET AL., 1991) eine untergeordnete Rolle spielt.

Eine Aktivität von Enterobakterien wird angenommen, die einen Teil des postileal anflutenden FC in Acylcarnitinester und nicht gemessene Produkte des bakteriellen L-Carnitin-Stoffwechsels wie γ -Butyrobetain, Trimethylamin und Trimethylamin-N-oxid umsetzt.

Die Differenz der gemessenen Mengen von FC, AC, PC und HC zwischen Exkrementen und Chymus ist in der Tab. 3 dargestellt. In der höchsten Zulagestufe scheinen die 4,7 mg FC, die praecaecal mehr anfluteten als mit den Exkrementen ausgeschieden wurden, primär in AC und PC verestert worden zu sein.

Tab. 3: Vergleich der täglichen Mengen an freiem und verestertem L-Carnitin in Exkrementen und Chymus beim Broiler nach unterschiedlich hoher L-Carnitin-Behandlung

L-Carnitin		freies	Acetyl-	Propionyl-	Hexanoyl-
Dosis [mg/kg]	Aufnahme [mg/d]				
L-Carnitin Differenz ¹ [mg/d]					
0	0	0,04	0,02	0,00	0,00
25	3	0,15	0,05	0,00	0,00
50	7	0,15	0,13	0,01	0,00
100	10	0,57	0,67	0,04	0,00
200	23	0,56	1,62	0,09	0,01
400	46	-4,67	4,01	0,22	0,01

¹ Mengen in Exkrementen minus Mengen im Chymus

Die Ester waren im Futter nicht enthalten und können im Gegensatz zum FC nur aus dem Stoffwechsel stammen. Es kann mit dieser Versuchsanstellung nicht gezeigt werden, ob die Ester aus enterobakteriellem Umsatz oder renalen Ausscheidungen stammen. Sollten die in Tab. 3 dargestellten Differenzen vollständig über die Niere reguliert worden sein, wäre der Anteil der renalen an der gesamten Ausscheidung für L-Carnitin beim Broiler trotzdem sehr gering.

Summary

It was the objective to determine precaecal net absorption of L-carnitine in broilers and to estimate the contribution of urine to total L-carnitine excretion. Six dietary levels of L-carnitine supplementation were used in a maize-soybean meal-based diet (mg/kg of diet: 0, 25, 50, 100, 200, and 400). Titanium dioxide was used as

indigestible marker. 360 chicks were penned in groups of 10 and fed the un-supplemented basal diet until d 21. Then, 6 pens were allocated to each of the 6 treatments and diets were offered ad libitum for 7 days. Spot samples of excreta were taken penwise during the last 3 days of the study. At the end all birds were asphyxiated with carbon dioxide and the section between Meckel's diverticulum and the ileo-caeco-colonic junction was removed and cut in three subsections of equal lengths and the content of each subsection from all birds of one pen pooled into one sample. Samples were homogenised, freeze dried, powdered and used for analysis of carnitine fractions.

Precaecal net absorption of L-carnitine was significantly different between the three subsections of the intestine and increased from proximal to terminal. Net absorption is, therefore, not completed at Meckel's diverticulum, and absorption is relevant even in the terminal ileum. Up to a level of 50 mg/kg of diet supplemented L-Carnitine was almost completely absorbed. Estimated urinary excretion was on an overall low level, which is in contrast to findings in mammals.

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Influence of supplemented Phytase on the Cu- and Zn-Content of pig carcasses

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Introduction

Zinc (Zn) and copper (Cu) are essential trace elements in the nutrition of plants and animals. In livestock production a deficiency of these elements can decrease animal performances and/or influence the health of animals (Mc Dowell 2003, Pallauf, 2003). Normally these elements are supplemented to compound feeds of livestock. In areas with a high livestock density, the excreted amounts of Zn and Cu lead to discussions of environmental problems (KTBL, 2002). This was the reason for the derivation of new upper limits of some trace elements in the EU recommendations valid from January 2004.

Especially when the supply of trace elements decreases because of environmental problems, the absorption rate (bioavailability) is of increasing interest. This bioavailability may be influenced by the phytate and/or phytase content of feed.

Material and Methods

50 carcasses from all castrated males (initial LW 25.5 ± 0.95 kg) from a trial with 100 growing-fattening pigs, (50 castrated males and 50 females) (Berk et al., 2003), were analysed to investigate the:

1. Influence of the level of Cu- and Zn-concentration in feed.
2. Influence of chemical binding form.
3. Supplementation of phytase (+700 units/kg feed) of microbial origin on Cu- and Zn-concentration in the whole body at about 115 kg LW.

There were three levels of feed concentration of the trace elements: native feed content only (7mg Cu/kg feed, 30 mg Zn/kg feed), and two levels of supplementation: (2 or 9 mg Cu/kg feed, Level 1, and 35 or 95 mg Zn/kg feed, Level 2) adequate to German requirements (GfE, 1987) and to the possible EU – upper levels.

The supplementations of Cu and Zn were given as sulphate (CuSO_4 and ZnSO_4) or as an amino acid – trace element – complex (AATEC).

All supplementation levels and trace element sources were added either with or without phytase (700 units/kg feed).

Consequently, the design was created as shown in Table 1.

Table 1. Experimental design

Group	1	2	3	4	5	6	7	8	9	10
n	10*	10*	10*	10*	10*	10*	10*	10*	10*	10*
Phytase	yes	yes	yes	yes	yes	no	no	no	no	no
Level	Native	1	1	2	2	Native	1	1	2	2
Source	Native	Inorg.	Org.	Inorg.	Org.	Native	Inorg.	Org.	Inorg.	Org.

*) 5 males/5 females

When reaching the slaughter weight of approximately 115 kg live weight (LW), these 50 animals were slaughtered, divided in the three fractions blood and offal (b+o), soft tissue, and bones of the left carcass half (corrected by the whole carcass weight). These fractions were analysed for DM, the contents of crude ash (CA), crude protein (CP), ether extract (EE) and of Cu and Zn.

Additionally liver, kidney and brain were analysed for DM and crude ash as well as Cu and Zn. The balances of the trace elements (Cu and Zn) were calculated on the basis of intake, the contents per kg LW, and the weight gain of the animals during the trial (from 25.5 kg to 111.2 kg LW) in the course of the experiment.

Results and discussion

The results of the performance are given by Berk et al. (2003). The mean live weight gain (LWG) was 891 ± 65 g/day for all 100 pigs, or 895 ± 67 of the slaughtered 50 pigs. This performance could be considered as normal but on a high level.

Analyses

The phytase contents analysed are shown in Table 2.

Table 2. Phytase content of the groups (FTU/kg feed)

Group	1	2	3	4	5	6 - 10
	1310	1650	1890	1860	1180	290

The content of 290 FTU/kg of the non supplemented feed is normal, corresponding to a wheat, barley and corn-based diet. The partly extreme high contents of the supplemented diets are inexplicable, but without any influence on the experimental questions.

The intake of Cu and Zn varied, depending on the “native” level on the one hand and the “highly supplemented” level on the other hand, from 1360 mg Cu and 6344 mg Zn up to 3816 mg Cu and 29840 mg Zn, respectively. The mean intake was 2237 ± 675 mg Cu, respectively 16156 ± 7319 mg Zn, per slaughtered animal (Tab. 3).

Table 3. Mean intake of feed (88% DM), Cu and Zn per animal during the growing period

Phytase supply	n	Feed (kg)	Cu (mg)	Zn (mg)
Yes	25	221 ± 20	2206 ± 662	15936 ± 7236
No	25	228 ± 22	2268 ± 700	16375 ± 7543

Considering all three factors of influence, the total intake of Cu and Zn is given in Table 4. The significant differences are due to the experimental design.

Table 4. Mean intake of Cu and Zn

Factor	n	Cu	Zn
Phytase yes	25	2206 ± 662	15936 ± 7236
no	25	2268 ± 700	16375 ± 7543
Source native	10	1583 ± 130 ^B	7034 ± 578 ^B
SO ₄	20	2575 ± 710 ^A	18857 ± 6795 ^A
AATEC	20	2256 ± 588 ^A	18327 ± 6125 ^A
Level native	10	1583 ± 130 ^C	7034 ± 578 ^C
1	20	1816 ± 180 ^B	12439 ± 962 ^B
2	20	2984 ± 368 ^A	24433 ± 2569 ^A

Tukey-Test, p < 0.05

Table 5. WBA of DM, CA, CP and EE (g/kg DM)

Phytase supply	n	DM	CA	CP	EE
Yes	25	405 ± 29	33 ± 2	149 ± 7	225 ± 33
No	25	413 ± 24	32 ± 2	152 ± 12	231 ± 31

Body Composition

There were no significant differences dependent on phytase supplementation in the whole body analysis (WBA) with regard to the contents of DM, CA, CP or EE (Tab. 5). The analyses of variance showed a significant influence of phytase for CA only ($Pr > F = 0.044$) for the criteria given in Table 4, and only a possible interaction of source x level ($Pr > F = 0.035$) for CP. The phytase supplementation led to significant differences in the amount of Cu in the fraction blood and offal (b+o), and the amount of Zn in the bones (Tab. 6).

Table 6. Influence of phytase supplementation on total amount of Cu/Zn (mg/animal)

Phytase supply	n	Soft tissue		Blood + offal		Bones	
		Cu	Zn	Cu	Zn	Cu	Zn
Yes	25	(49±10)	1161±112	25±3 ^B	219±40	17±19	515±88 ^A
No	25	(49±12)	1168±133	29±6 ^A	203±24	11±5	434±110 ^B

t-Test ($p < 0.05$); values in brackets means possible interaction phytase x source ($Pr > F = 0.029$)

Investigated Organs

It should be mentioned that in the case of the fraction b+o, the phytase supplementation lead to a lower amount of Cu and to higher amount of Zn. That is also true if comparing the contents of these trace elements in the liver (Tab. 7).

Table 7. Influence of phytase supplementation on Cu/Zn concentration in organs (mg/kg DM)

Phytase supply	n	Brain		Kidney		Liver	
		Cu	Zn	Cu	Zn	Cu	Zn
Yes	25	11±2	48±4	21±6 ^A	94±11 ^A	(20±4) ^B	213±80 ^A
No	25	12±2	49±5	17±4 ^B	85±11 ^B	(27±10) ^A	169±51 ^B

t-Test ($p < 0.05$); values in brackets means possible interaction phytase x source ($Pr > F = 0.012$)

No significant difference exists in the contents of both trace elements in the brain. But all other concentrations are significantly different. In the cases of Cu and Zn in the kidney, and Zn in the liver, the contents are higher if phytase is added to the feed. In contrast, in the case of Cu in the liver, the content is lower when phytase was added.

But an interaction was analysed for the source of supplementation. This could probably be a reason for these unexpected results. No significant differences in the trace element concentrations of organs were measured due to trace element source (Tab. 8).

Calculation of Balance

Especially the results of the kidney values indicate that there is an influence of absorption of nutrients, but in the case of Cu or Zn, the animals had no additional need and so these elements were excreted. Comparing the total balance of the whole body and the resulting excretion in the case of phytase supplementation, one can see that there is, in contrast to the values of the kidney investigation, a numerically higher balance of both elements. This consequently leads to a numerically lower excretion of both investigated trace elements (Tab. 9).

Table 8. Influence of source of supplementation on Cu/Zn concentration in organs (mg/kg DM)

Source	n	Brain		Kidney		Liver	
		Cu	Zn	Cu	Zn	Cu	Zn
Native	10	12±2	48±5	17±4	83±6	(23±5)	150±56
SO ₄	20	12±3	49±4	20±7	91±15	(26±11)	197±69
AATEC	20	11±2	49±4	19±4	91±9	(22±5)	205±73

Tukey-Test ($p < 0.05$); values in brackets means possible interaction phytase x source ($Pr > F = 0.012$)

Table 9. Influence of phytase supply on Cu/Zn balance and excretion (mg/animal)

Phytase supply	n	Total amount		Balance		Excretion	
		Cu	Zn	Cu	Zn	Cu	Zn
Yes	25	91	1896	70	1456	2096	14480
		±25	±173	±19	±139	±706	±7182
No	25	89	1805	68	1394	2200	14981
		±12	±205	±9	±161	±701	±7469

t-Test ($p < 0.05$)

Table 10. Influence of level of supply on Cu/Zn balance and excretion (mg/animal)

Level	n	Balance		Excretion	
		Cu	Zn	Cu	Zn
Native	10	71±26	1311±163 ^B	1512±137 ^B	5723±499 ^C
1	20	68±11	1431±137 ^{AB}	1699±262 ^B	11008±944 ^B
2	20	69±11	1477±138 ^A	2915±365 ^A	22956±2500 ^A

Tukey-Test (p < 0.05)

The first level of supplementation of the trace elements Cu and Zn showed an insignificantly higher balance in the whole body of the investigated animals, but in the case of Zn, a significantly higher excretion. Only the second level led to a significantly higher balance of Zn, but a numerically lower balance in the case of Cu. The excretion of the second level is significantly higher for both elements of the native variant (Tab. 10).

In the case of Level 2, the excretion reached nearly double the level of the native groups (Cu), and actually double that of the first level groups (Zn) (p < 0.05).

Summary

The supplementation of phytase to cereals–soy bean meal–diets did not significantly influence pig performance of growing – finishing pigs from 25 to 115 kg LW (p > 0.05) (Berk et al., 2003). But the total balance of Cu and Zn is numerically higher, and the resulting excretion is numerically lower when phytase is added to the feed, because of the higher Cu (numerically) and Zn (significantly) amount in the fraction bones. The significantly (p < 0.05) higher amounts of Cu and Zn in the kidneys of animals supplemented with phytase indicate no effect concerning a higher transfer in the body tissue.

Like the performance data, the whole body analysis data showed clearly that the German recommendations (GfE, 1987) (Level 1) concerning Zn and Cu are safe enough to achieve a good health combined with high pig performance.

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Influence of different non starch polysaccharide degrading feed enzymes on the intestinal microbiota in piglets

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Introduction

Like any other microbial habitat, the microbiota of the porcine intestinal tract is largely determined by amount and type of substrates available. Dietary carbohydrates are the main energy sources for intestinal bacteria, however, due to an efficient starch digestion by the host the bacteria can only ferment structural carbohydrates, namely non-starch-polysaccharides (NSP). Soluble NSP are known as antinutritive components in cereal based broiler chicken diets and NSP degrading enzymes have been used successfully to circumvent soluble NSP related viscosity problems. However, the influence of soluble NSP on digestion in piglets is less clear. Soluble NSP have been shown to be fermented in the porcine small intestine to a rather large extent and thus, the addition of NSP degrading enzymes may further modify bacterial populations. This study was carried out to investigate the modifications of a mono enzyme preparation and a multi enzyme preparation on the intestinal microbiota in weaned piglets.

Material and Methods

Feeding trial

Animals, experimental design and diets

Weaned piglets (n = 20) were allocated to boxes in a temperature and light controlled room. Feed was offered in two times daily in mash form. Details of the experimental design are shown in table 1. The composition and calculated nutrient contents are shown in table 2.

Table 1: Experimental design

Treatment (mg/kg)	A	B	C
Multienzyme*	0	100	-
Monoenzyme**	0	-	200
Replicates	20	20	20

* 3000 IE/g 1,4- β -Arabinoxylanase, 2800 IE/g 1,3-1,4- β -Glucanase, 140 IE/g 1,4- β -Arabinogalactanase, 190 IE/g 1,4- β -Galactomannanase, 90 IE/g 1,3- β -Glucanase, 10 IE/g 1,4- β -Polygalacturonase; 16 IE/g Cellulase

** 1200 IE/g Xylanase

Sampling

Samples of the stomach, central jejunum, ileum and colon ascendens were taken after slaughter, divided into subsamples for respective analyses and frozen at – 80 °C until further analysis. Fresh digesta supernatants were used for the analysis of enzyme activities.

Table 2: Composition of basal diet and calculated nutrients

Composition (g/ kg)	
Wheat	72.42
Wheat Bran	10.70
Soyabean meal 48 CP	1.00
Potato protein	10.70
Limestone	1.97
Soya oil	1.00
Sunflower meal	4.00
Premix*	1.20
Monocalciumphosphate	1.79
Methionine	0.17
Lysine	0.22
Calculation	
ME _{BFS} (MJ/kg)	10.97
Starch	353.70
Sugars	30.60
Calcium	34.00
Phosphorus	5.00
Sodium	1.92
Analysis (g/ kg)	
Crude protein	205.35
Crude fat	19.08
Crude fiber	39.20
ADF	51.48
NDF	139.35
Lysine	10.43
Methionine	2.85
Cystine	3.42

In vitro assays on bacterial growth

A minimal medium supplemented with a single carbohydrate source was used to grow bacteria in samples of the stomach, central jejunum and colon ascendens. Carbohydrate substrates (1% (w/v)) consisted of either arabinoxylan from wheat (Megazyme, Ireland), lichenan from algae (Sigma, Deisenhofen, Germany), water soluble extract of the wheat batch used in the feeding trial or ultrafiltrated wheat extract (30 kD cutoff). The media were preincubated with a 1mg/ ml sterile filtrated enzyme solution (1h, 37 °C). Microtiter plates were sealed with parafilm and

incubated for 24 h at 37 °C. Optical density at 690 nm was recorded and controls (0h, uninoculated controls) were subtracted to calculate bacterial growth.

Results

In vitro assays

Compared to the controls, the presence of the monoenzyme in the arabinoxylan supplemented medium led to growth reduction in all digesta samples (table 4). The multienzyme also reduced growth in stomach samples, however jejunal and colon samples showed higher bacterial growth. As expected, no differences in growth were observed with the lichenan medium and added monoenzyme (table 5). The trend for growth reduction in stomach and in part jejunum samples was also visible in incubations with the wheat extract (table 6).

Table 4: Bacterial growth of piglet digesta in media supplemented with arabinoxylan preincubated with the monoenzyme or the multienzyme [OD_{690nm}]¹

Enzyme	Stomach		Jejunum		Colon	
	Enzyme	Buffer	Enzym	Buffer	Enzym	Buffer
Multi	0.02 (±0.01)	0.07 (± 0.01)	0.41 (±0.2)	0.31 (±0.07)	0.25 (±0.06)	0.17 (±0.14)
Mono	0	0.03 (±0.05)	0.18 (±0)	0.30 (±0.02)	0.02 (±0.05)	0.10 (±0.07)

¹ = 1mg/ml final concentration

Table 5: Bacterial growth of piglet digesta in media supplemented with lichenan preincubated with the monoenzyme or the multienzyme [OD_{690nm}]¹

Enzyme	Stomach		Jejunum		Colon	
	Enzyme	Buffer	Enzym	Buffer	Enzym	Buffer
Multi	0.95 (± 0.04)	0.67 (± 0.01)	0.91 (±0.1)	n.d.	0.55 (±0.02)	0.23 (±0.06)
Mono	0	n.d.	0.2 (±0.06)	0.20 (±0.05)	0.13 (±0.02)	0.14 (±0.01)

¹ = 1mg/ml final concentration

n.d. = not determined

Table 6: Bacterial growth of piglet digesta in media supplemented with wheat extract preincubated with the monoenzyme or the multienzyme [OD_{690nm}]¹

	Stomach		Jejunum		Colon	
	Enzyme	Buffer	Enzyme	Buffer	Enzyme	Buffer
Multi	0	0.20 (±0.05)	0.69 (±0.01)	0.93 (±0.03)	0.24 (±0.03)	0.08 (±0.06)
Mono	0.03 (±0.01)	0.14 (±0.04)	0.89 (±0.02)	0.91 (±0.05)	0.35 (±0.01)	0.08 (±0.03)

¹ = 1mg/ml final concentration

Interestingly, incubations with the ultrafiltrated fraction of the wheat extract showed only minor differences in growth (table 7).

Table 7: Bacterial growth of piglet digesta in media supplemented with ultrafiltrated wheat extract (30 kD cutoff) preincubated with the monoenzyme or the multienzyme [OD_{690nm}]¹

	Stomach		Jejunum		Colon	
	Enzyme	Buffer	Enzyme	Buffer	Enzyme	Buffer
Multi	0.01 (±0.01)	0.04 (±0.01)	0.78 (±0.05)	0.69 (±0.06)	0.51 (±0.02)	0.47 (±0.06)
Mono	0.01 (±0.01)	0.03 (±0.01)	0.78 (±0.05)	0.88 (±0.07)	0.43 (±0.01)	0.49 (±0.05)

¹ = 1mg/ml final concentration

Feeding trial

Performance

The multienzyme and the monoenzyme showed nonsignificantly increased ADG by 6.8 and 7.1 %, respectively (table 8). Animals fed the enzyme supplemented diets also showed a higher feed consumption, resulting in an increased feed conversion for the multienzyme group. The FCR was improved in the monoenzyme supplemented group. Differences were especially high during the first week of the trial (see appendix A).

Table 8: Summary of performance of weaned piglets fed diets supplemented with the multienzyme or the monoenzyme from week 1 to week 4

	Control	Multienzyme	Monoenzyme
ADG	8.40±1.40	8.97±1.70	9.00±1.10
Relative	100	106.8	107.1
Feed consumption	11.53±1.80	12.49±2.15	12.09±1.53
Relative	100	108.3	104.9
Feed conversion	1.376±0.062	1.398±0.086	1.345±0.097
Relative	100	101.6	97.7

Table 9: Intestinal viscosity in weaned piglets fed diets supplemented with the monoenzyme or the multienzyme

	Stomach	Jejunum	Colon
Control	1.12 (±0.12)	11.9 (±5.7) ^a	16.3 (±20.6) ^a
Multienzyme	1.20 (±0.11)	5.6 (±3.6) ^b	12.3 (±13.3) ^a
Monoenzyme	1.15 (±0.31)	2.9 (±0.4) ^b	3.1 (±1.4) ^b

a,b = significantly different within a column

Intestinal viscosity

A clear influence of enzyme supplementation on intestinal viscosity was found in the jejunum (table 9).

Lactate concentrations

Significant differences ($p < 0.05$) were found for intestinal lactate in the stomach for the multienzyme preparation (table 10). The multienzyme tended to increase lactate concentrations in the colon.

Table 10: Lactate concentrations in the intestine of piglets fed diets supplemented with the monoenzyme or the multienzyme [mmol/l]

	Stomach	Jejunum	Colon
Control	0.52 (± 0.10) ^a	0.55 (± 0.37)	0.1 (± 0.09) ^a
Multienzyme	0.17 (± 0.08) ^b	0.50 (± 0.15)	0.2 (± 0.15) ^a
Monoenzyme	0.39 (± 0.31) ^a	0.43 (± 0.23)	0.07 (± 0.07) ^b

a,b = significantly different within a column

Volatile fatty acids

Volatile fatty acid concentration in colon samples are presented in table 11. Both enzymes increased total VFA.

Table 11: Volatile fatty acid concentrations in the intestine of piglets fed diets supplemented with the monoenzyme or the multienzyme [mmol/l]

	Control	Multienzyme	Monoenzyme
Total	80.7 (± 40.9) ^a	105.6 (± 8.4) ^b	112.0 (± 11.4) ^b
Acetate	57.8 (± 8.0) ^a	63.6 (± 6.3) ^{ab}	68.7 (± 7.2) ^b
Propionate	26.4 (± 4.5) ^a	27.7 (± 5.5) ^a	30.3 (± 8.9) ^b
n-butyrate	10.2 (± 1.3)	11.3 (± 2.1)	10.1 (± 2.4)
i-butyrate	0.43 (± 0.35) ^a	0.35 (± 0.08) ^b	0.48 (± 0.12) ^a
n-valerate	1.7 (± 0.84)	2.3 (± 1.0)	2.0 (± 0.78)
i-valerate	0.54 (± 0.18)	0.40 (± 0.09)	0.53 (± 0.31)

a,b = significantly different within a row

Ammonia concentrations

Free ammonia concentrations in the stomach were not different between groups (data not shown). However, jejunal samples showed a trend for decreased ammonia in the multienzyme group, but increased concentrations in the monoenzyme group compared to the control. Both enzymes increased ammonia in the colon.

NSP degrading enzyme activities

NSP degrading enzyme activities in the jejunum and colon in part reflected the respective main enzyme activities of the supplemented enzyme preparations,

however the control group also displayed high activity. Increased xylanase activity was observed in digesta supernatants of animals fed enzyme supplemented diets, most pronounced in the monoenzyme supplemented group (table 12).

Table 12: Xylanase and lichenase activities in the intestine of piglets fed diets supplemented with the monoenzyme or the multienzyme [mm² lysis zone]

	Control		Multienzyme		Monoenzyme	
	Xylan	Lichenan	Xylan	Lichenan	Xylan	Lichenan
Jejunum	478 (±260)	540 (±175)	542 (±161)	712 (±153)	563 (±195)	435 (±84)
Colon	1531 (±173)	762 (±146)	1504 (±207)	760 (±165)	1635 (±256)	819 (±151)

Discussion

In vitro studies with piglet digesta have shown that intestinal bacteria react depending on intestinal location and supplemented enzyme. Growth inhibition in the small intestine involves lactic acid bacteria (LAB) as the main population. Additionally, *in vivo* results show that lactate and volatile fatty acids correlated negatively for both enzyme supplemented groups. A shift from lactate to acetate production in the small intestine indicates either a general reduction of LAB or a metabolic change of heterofermentative bacteria switching to acetate production. Furthermore, the growth of a few specific bacterial genera which inhibit other bacteria may also have occurred. In a similar study with the mono enzyme preparation and a wheat based diet it was shown that the heterofermentative *Lactobacillus reuteri* dominated the small intestine of weaned piglets (Zimprich et al., 2001).

The increased growth in the colon can be explained by increasing use of breakdown of NSP- substrates by the enzymes. However, arabinoxylan fragments generated by the monoenzyme were not responsible for the promotion of bacterial growth in the colon, as only the wheat extract showed increased growth *in vitro*.

Fermentation of available substrates seemed most pronounced in the monoenzyme preparation, however composition of metabolites indicates a different microbiota depending on the nature of the supplemented enzyme.

Literature

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Effect of calcium level, microbial phytase and citric acid on laying performance, bone mineralization and phosphorus disappearance in the digestive tract of laying hens fed corn soybean meal diets

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Introduction

Calcium is reported to have a negative effect on efficiency of phytase, even at high phytase levels (Boling et al., 2000). Published data are scanty on the efficacy of microbial phytase at different levels of dietary calcium in laying hens. Therefore one of the objectives of this study was to determine the effect of supplemental microbial phytase at two different levels of dietary calcium on the performance parameters and phosphorus disappearance from the digestive tract of laying hens. Sukria and Liebert (2002) reported in growing chicken that citric acid has the ability to improve the efficiency of microbial phytase. Afsharmanesh and Pourreza (2005) also reported that adding microbial phytase in combination with citric acid improved growth in chicken. However, the level of dietary calcium added to layer diets is much higher than in growing chicken. Therefore, the second objective of the current study was to investigate whether the combination of microbial phytase and citric acid could improve the efficacy of microbial phytase in laying hens fed diets with reduced calcium concentration.

Materials and Methods

Diets (Table 1) were factorially arranged as two levels of calcium (3.8% and 2.6%), two levels of phytase (0 and 300 FTU/Kg) and two levels of citric acid (0 and 2.5%). Seventy two LOHMAN BROWN-CLASSIC laying hens were randomly assigned to one of eight dietary treatments in an experiment that was conducted from 23 to 38 wk of age (n = 9). The hens were kept in individual cages with the stocking density of 6.9 hens/m². The birds were housed in a completely enclosed, ventilated cage layer system in which they were exposed to a 14 h light: 10 h dark daily lighting schedule. The composition of corn-SBM basal diets is shown in Table 2. Total P was kept constant in all dietary treatments at 0.33% (0.12% Non phytate P). Other dietary nutrients were formulated to meet nutrient requirements of laying hens (NRC, 1994).

The diets were presented in mash form; feed and water were consumed *ad libitum*. The utilized microbial phytase RONOZYME-P5000® (Roche Vitamins Ltd., Basel, Switzerland), is a 6-phytase from *Peniophora lycii* with an activity of 5000 units (FTU) per gram. Egg production and egg mass were daily determined. Feed Intake was recorded weekly. Final body weight and feed conversion ratio were calculated. At the end of the experiment, the individual hens were slaughtered, the left tibia was removed and cleaned of all adhering tissue. Bones were dried at 105 °C for two days, dry-ashed at 550 °C to determine the tibia ash weight. The Ca in tibia ash was determined using an atomic absorption spectrophotometer at a wavelength of 422.7 nm. P in tibia ash was analyzed according to VDLUFA method (Naumann and Bassler, 1976-1997). One week before finishing the experiment, 0.2% titanium dioxide (TiO₂) was added to the feed. The birds were starved over night, given free access to the feed for 4 hours and slaughtered. Digesta samples were squeezed manually from the crop and the proximal small intestine. Titanium was measured with an atomic absorption spectrophotometer at a wavelength of 364.3 nm. Phosphorus disappearance in the digestive tract was estimated by the following formula:

$$D_P = 100 - [(TiO_2\%)_{diet} : (TiO_2\%)_{digesta} \times (total\ P\%)_{digesta} : (total\ P\%)_{diet} \times 100]$$

Where: D_P : P Disappearance (%)

$(TiO_2\%)_{diet}$: concentration of titanium dioxide in the diet

$(TiO_2\%)_{digesta}$: concentration of titanium dioxide in the digesta

$(total\ P\%)_{digesta}$: concentration of phosphorus in the digesta

$(total\ P\%)_{diet}$: concentration of phosphorus in the diet.

Table 1: Experimental design

Diet	Ca-level (%)	Phytase supplemented* (FTU/kg)	Citric acid added (%)
T1	3.8	0	0
T2	3.8	300	0
T3	3.8	0	2.5
T4	3.8	300	2.5
T5	2.6	0	0
T6	2.6	300	0
T7	2.6	0	2.5
T8	2.6	300	2.5

*RONOZYME-P5000®

Data were subjected to ANOVA using the general linear model procedure of SPSS program (10.0). Significant differences among treatment means were assessed with the LSD test at $P \leq 0.05$.

Table 2: Ingredients and nutrient composition of the experimental diets

Ingredients (%)	3.8% Ca (T1 – T4)	2.6% Ca (T5 – T8)
Corn	57.23	57.23
Soybean meal (49)	24.15	24.15
Soybean oil	3.10	0.59
Wheat starch	2.62	8.56
CaCO ₃	9.90	6.52
NaCl	0.05	0.05
Cellulose powder	1.85	1.80
DL-Methionine	0.10	0.10
Premix ¹	1.00	1.00
Nutrient composition (g/kg 90%DM)		
Crude protein	162.1	162.1
Crude fat	32.8	32.8
Crude ash	124.9	93.5
Calcium (total)	38.46	27.22
Phosphorus (total)	3.24	3.24
Phytate-P	2.25 ²	2.25
Metabolizable Energy (MJ)	11.36	11.36

¹ 1 Kg of Premix contains: 600,000 I.U Vitamin A, 100,000 I.U vitamin D3, 1,850 mg vitamin E, 160 mg vitamin B1, 480 mg vitamin B2, 500 mg vitamin B6, 2,000 mcg vitamin B12, 200 mg vitamin K3, 2800 mg Nicotinic acid, 1000 mg Ca-Pantothenat, 60 mg folic acid, 10000 mcg Biotin, 80000 mg cholinchlorid, 2500 mg Fe, 1600 mg Cu, 8000 mg Mn, 8000 mg Zn, 120 mg I, 25 mg Se, 55 mg Co, 10000 mg B H T, 350 mg Canthaxanthin. ² 69.4% of total P

Results and Discussion

Egg production (EP), egg mass (EM), feed intake (FI), feed conversion ratio (FCR) and body weight (BW) for the entire 23rd to 38th-wk period are shown in Table 3. Due to statistical analysis of the main effect (not included in table 3), egg production (%) and daily egg mass were significantly reduced at low calcium level (2.6%) compared to hens fed high Ca level (3.8%). Egg production and daily egg mass were not affected by supplementation of phytase and/or citric acid. At both levels of calcium, adding citric acid decreased the feed intake ($P = 0.058$). The decrease in feed intake from citric acid was probably due to decreasing the feed palatability. The final body weight of hens fed high calcium diets (3.8%) was higher compared to low calcium

diets (2.6%). Citric acid supplementation reduced the body weight at both calcium levels ($p = 0.004$) due to depression of feed intake. Phytase supplementation (without citric acid) tended to increase ($P = 0.083$) body weight (T2 vs. T1 and T6 vs. T5). Low Ca diets impaired feed efficiency. At both calcium levels, adding citric acid significantly improved feed efficiency (T1 vs T3 and T5 vs. T7). This improvement of feed efficiency can not be over interpreted but is due to increased mobilization and loss of body weight.

Table 3: Zootechnical parameters (23rd-38th week)

Diet	Ca-level (%)	Phytase supplemented (FTU/kg)	Citric acid added (%)	BW ¹	EP ²	EM ³	FI ⁴	FCR ⁵
				g	%	g/d	g/d	g/g
T1	3.8	0	0	1921 ^{ab}	96.58 ^{ab}	58.07 ^{ab}	114.1 ^a	1.96 ^{bc}
T2	3.8	300	0	1983 ^a	98.18 ^a	60.94 ^{ab}	117.3 ^a	1.92 ^c
T3	3.8	0	2.5	1752 ^{cd}	97.43 ^{ab}	61.63 ^a	110.0 ^a	1.78 ^d
T4	3.8	300	2.5	1899 ^{ab}	97.33 ^{ab}	59.31 ^{ab}	111.2 ^a	1.87 ^{cd}
T5	2.6	0	0	1782 ^{bcd}	92.55 ^b	56.01 ^b	114.8 ^a	2.06 ^{ab}
T6	2.6	300	0	1860 ^{abc}	94.70 ^{ab}	56.04 ^b	117.3 ^a	2.09 ^a
T7	2.6	0	2.5	1647 ^d	93.12 ^b	55.80 ^b	107.6 ^a	1.93 ^c
T8	2.6	300	2.5	1687 ^{cd}	97.03 ^{ab}	58.16 ^{ab}	115.2 ^a	1.98 ^{abc}
SE				92.91	2.53	2.3	5.05	0.062

^{a, b, c} Means within a column with no common superscript letters significantly different ($P < 0.05$).

¹BW: Final Body weight, ²EP: Egg production, ³EM: Egg mass, ⁴FI: Feed intake, ⁵FCR: Feed conversion ratio.

Table 4: Effects on tibia bone mineralization

Diet	Ca-level (%)	Phytase supplemented (FTU/kg)	Citric acid added (%)	Ash in tibia	Ca in tibia	P in tibia
				(%)	(%)	(%)
T1	3.8	0	0	54.56 ^{bc}	33.13 ^{bcd}	15.70 ^{cd}
T2	3.8	300	0	58.29 ^a	35.26 ^a	16.06 ^a
T3	3.8	0	2.5	54.57 ^{bc}	33.60 ^b	15.95 ^{ab}
T4	3.8	300	2.5	56.66 ^{ab}	33.00 ^{cd}	15.60 ^{cd}
T5	2.6	0	0	52.88 ^{cd}	32.20 ^e	15.50 ^d
T6	2.6	300	0	53.99 ^{bc}	33.39 ^{bc}	15.77 ^{bc}
T7	2.6	0	2.5	50.14 ^d	32.82 ^d	16.09 ^a
T8	2.6	300	2.5	54.58 ^{bc}	32.67 ^{de}	15.66 ^{cd}
SE				1.64	0.282	0.119

^{a, b, c} Means within a column with no common superscript letters significantly different ($P < 0.05$).

Tibia ash and calcium in tibia ash were significantly reduced due to low calcium diets (Table 4). Supplementing diets with microbial phytase increased tibia ash content ($p=0.001$). Adding citric acid without phytase supplementation in low calcium diet (T7) significantly increased calcium in tibia ash compared to T5. Due to least significant differences test between individual treatments, adding microbial phytase alone (T2, T6) at the both levels of calcium increased P in tibia ash.

Phosphorus disappearance in the digestive tract

Phosphorus disappearance from the crop content was significantly higher at low calcium diets (T1 vs. T5). At both calcium levels phytate P concentration was lower due to phytase supplementation (T1 vs. T2 and T5 vs. T6). Adding microbial phytase in combination with citric acid did not show a further significant degradation of phytate P in the crop (T2 vs. T4 and T6 vs. T8).

Table 5: Effects on total P disappearance in the crop.

Diet	Ca %	Phytase (FTU/kg)	Citric acid %	Phytate P% from the total P %	tP disappearance from the crop %
T1	3.8	0	0	73.9 ^{bc}	0.83 ^e
T2	3.8	300	0	54.6 ^e	13.44 ^a
T3	3.8	0	2.5	73.7 ^{bc}	9.91 ^{ab}
T4	3.8	300	2.5	63.1 ^{de}	3.33 ^{de}
T5	2.6	0	0	82.1 ^{ab}	9.56 ^{bc}
T6	2.6	300	0	72.3 ^{bcd}	11.30 ^{ab}
T7	2.6	0	2.5	85.3 ^a	8.45 ^{bc}
T8	2.6	300	2.5	69.2 ^{cd}	6.32 ^{cd}
SE				4.70	0.82

^{a, b, c, d} Means within a column with no common superscript letters significantly different ($P<0.05$).

Microbial phytase increased the phosphorus disappearance from the crop content ($P = 0.08$). As a main effect, citric acid did not show a significant effect on the P disappearance in the crop ($P = 0.09$).

Summary

A 38 week trial was conducted to investigate the effect of calcium, microbial phytase and citric acid on the performance parameters and bone mineralization in laying hens fed corn soybean meal diets. A total of 72 LOHMAN BROWN-CLASSIC laying hens, 23 wk of age were randomly assigned into a 2×2×2 factorial arrangement of treatments with two levels of calcium (2.6 and 3.8%), two levels of phytase (0 and 300 FTU/kg) and two levels of citric acid (0 and 2.6%). The phosphorus level was 0.33% tP (0.12% Non-phytate phosphorus) in all dietary treatments. Body weight, egg production, egg mass and feed conversion ratio were significantly reduced by feeding 2.6% calcium, adding microbial phytase did not prevent the adverse effect of low calcium diets (2.6%). Tibia ash and calcium in tibia ash were increased by adding microbial phytase. Feed conversion ratio, and calcium and phosphorus in tibia ash were improved by adding citric acid, whereas body weight and feed intake were decreased. Phosphorus disappearance of the crop contents was reduced when hens were fed high calcium diets (3.8%) compared to hens which were fed low calcium diets (2.6%).

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Calcium and phosphorus intake, apparent absorption, balance and normative requirement of adults – Are supplementations necessary?

Verzehr, scheinbare Absorption, Bilanz und Bedarf von Calcium und Phosphor Erwachsener – Sind Supplementationen erforderlich?

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Der normative Calciumbedarf der Frau wurde nach zahlreichen Duplikat- und Bilanzstudien auf 500 mg / Tag, der Stillender auf 750 mg / Tag und der Männer auf 600 mg / Tag im Wochenmittel veranschlagt. Ein Calciumverzehr Erwachsener von 9 mg/kg Körpergewicht, wie er im Mittel, aber nicht individuell bei Mischköstlern gegeben ist, deckt den normativen Calciumbedarf Erwachsener. Vegetarier (Ovolaktovegetarier) konsumieren je kg Körpergewicht die doppelte Calciummenge wie Mischköstler, wobei die Bioverfügbarkeit des pflanzlichen Calciums niedriger ist (*Anke et al. 2002*). Die Deutsche Gesellschaft für Ernährung der Schweiz und Österreichs empfehlen Erwachsenen den Konsum von 1000 mg Ca / Tag (*Anonym 2000*). Erstaunlicherweise ist der normative Phosphorbedarf des Menschen unbekannt. Die WHO-FAO teilen für den Phosphorbedarf des Menschen keinen Richtwert mit (*Parr et al. 1992*), obwohl sie viele Einzelwerte der Phosphoraufnahme in allen Teilen der Welt publizieren. Die Ernährungsgesellschaften Deutschlands, Österreichs und der Schweiz empfehlen Frauen und Männern den Verzehr von 700 mg P / Tag, Schwangeren von 800 und Stillenden von 900 mg / Tag (*Anonym 2000*). Aufgabe der Untersuchungen war es, den Verzehr von Calcium und Phosphor erwachsener Mischköstler in Deutschland zu bestimmen, deren scheinbare Absorption zu ermitteln und vergleichende Schlussfolgerungen für möglicherweise gegebene Notwendigkeiten der Ergänzung mit Calcium und/oder Phosphor zu geben.

Material und Methoden

Für die Unternehmen standen 10 deutsche Testpopulationen von jeweils 7 Mischköstlerinnen und 7 Mischköstlern im Alter von 20 bis 69 Jahren zur Verfügung, die an 7 aufeinander folgenden Tagen alle Speisen, Naschereien und Getränke tageweise sammelten. Dazu kamen 2 Teams von Studentinnen, die in einer plazebokontrollierten Doppelblindstudie zusätzlich 500 mg Ca / Tag bekamen und 2 Gruppen schwangerer und stillender Frauen, die gleichermaßen 500 mg Ca / Tag zusätzlich vom 8. Schwangerschaftsmonat bis zum Ende des Stillens aufnahmen bzw. auf diese Ergänzung verzichteten.

Bei 3 der 10 deutschen Testkollektive war es möglich, neben den Duplikaten die Ausscheidungen (Urin, Fäzes) tageweise zu sammeln. Das gleiche gilt für die 2 Gruppen der Studentinnen mit unterschiedlicher Calciumaufnahme und die Stillenden. Von letzteren wurden neben Harn und Kot auch Milchproben genommen. *Tabelle 1* informiert über den Umfang des gesammelten und analysierten Probenmaterials. Die Analyse des Calciumgehaltes erfolgte einerseits mittels Flammenatomspektroskopie, wobei die den gelben Phosphovanadomolybdatkomplex enthaltenden, für die Phosphorbestimmung vorbereiteten Probenlösungen in einer Luft-Azetylen-Flamme zerstäubt wurden (*Sarudi und Varga 1982*) und andererseits mittels ICP-OES (Spectroflame D, Spectro Analytical Elements Kleve, Deutschland). Der Fehler der Calciumbestimmung beträgt $\pm 5\%$. Die Richtigkeit der Analyse wurde mittels des Referenzmaterials „ARC / CI total diet reference material (HDP)“ ermittelt.

Table 1: Number of the analyzed samples

Kind of samples	table	Ca	P
Duplicate samples, 10 test populations	1, 2	490	490
Duplicate samples, students	7	98	0
Duplicate samples, breast feeding	7	98	0
Urine and faeces samples, 3 test teams	4, 5, 6	294	294
Urine and faeces samples, students	7	196	0
Urine, faeces and milk samples, breast feeding	7	203	0
Foodstuffs, people with mixed diets	3	1658	1658
Number of samples (n)	–	3037	2442

Ergebnisse

1. Der Calcium- und Phosphorverzehr Erwachsener

In Deutschland konsumierten Mischköstlerinnen im Mittel der 10 Testteams ≈ 500 und Mischköstler ≈ 650 mg Ca / Tag im Wochenmittel (*Tabelle 2*). Ihr mittlerer Calciumkonsum entsprach dem normativen Calciumbedarf Erwachsener, ohne individuell diesen in jedem Fall zu befriedigen.

Table 2: Calcium and phosphorus intake of German adults with mixed diets

Parameter (n;n)	Women		Men		p ¹⁾	%
	s	x	x	s		
Calcium (490;490) / (471;504)	277	512	667	383	< 0.001	130
Phosphorus (490;490) / (471;504)	307	810	1056	393	< 0.001	130
p	< 0.001		< 0.001		-	
Ca:P ratio	1:1.58		1:1.58		-	

¹⁾ Significance level (Student t-test)

Die Aufgliederungen des Calciumverzehrs auf die 10 verschiedenen Lebensräume belegt die regionale Varianz der Calciumaufnahme der Frauen von 390 bis 628 mg / Tag im Wochenmittel und die der Männer von 460 bis 798 mg / Tag (*Tabelle 3*). Die Bewohner des Thüringer Waldes (Bad Liebenstein) und der norddeutschen, diluvialen Sande (Wusterhausen A, B; Vetschau) verzehrten besonders wenig, die der Muschelkalk-verwitterungs- und Keuperstandorte des Trias (Bad Langensalza A, B; Jena) reichlich Calcium. Individuell nahmen 38 % der Frauen weniger als 500 mg Ca / Tag im Wochenmittel (*Abbildung 1*) und 52 % der Männer < 600 mg / Tag auf (*Abbildung 2*) und deckten damit ihren normativen Calciumbedarf nicht.

Der Phosphorkonsum der Mischköstler übersteigt den Calciumverzehr um > 50 % (*Tabelle 2*). Das Verhältnis von Calcium zu Phosphor in der aufgenommenen Nahrung erreicht im Mittel ein Verhältnis von 2:3,2, obwohl beide Elemente zum Aufbau des Skelettes ein solches von 2 Teilen Calcium und 1 Teil Phosphor benötigen. In den zehn verschiedenen Lebensräumen schwankte das Verhältnis Calcium:Phosphor in der verzehrten Nahrung zwischen 1:1,36 bis 1:1,93 oder 2:2,72 bis 2:3,86.

Die von den Frauen und Männern mit der Mischkost aufgenommenen Phosphormengen sind im Mittel und individuell beträchtlich zu hoch. Die Erwachsenen zur Aufnahme empfohlene Phosphormenge von 700 mg / Tag übersteigt die in das Skelett einzubauende Phosphormengen erheblich.

Table 3: Calcium and phosphorus intake of adults with mixed diets in mg / day

Habitat	Women			Men		
	Calcium	Phosphorus	Ca:P	Calcium	Phosphorus	Ca:P
Bad Liebenstein	390	694	1:1.78	473	862	1:1.82
Wusterhausen, A	400	839	1:2.10	693	1192	1:1.72
Wusterhausen, B	426	712	1:1.67	713	1094	1:1.53
Vetschau	439	780	1:1.78	460	890	1:1.93
Chemnitz	530	813	1:1.53	670	1212	1:1.81
Greifswald	554	818	1:1.48	627	909	1:1.45
Freiberg	557	804	1:1.44	679	1072	1:1.58
Bad Langensalza, A	591	775	1:1.31	745	1014	1:1.36
Jena	627	955	1:1.52	798	1164	1:1.46
Bad Langensalza, B	628	873	1:1.39	777	1167	1:1.50
Arithmetic mean	512	810	1:1.58	667	1056	1:1.58

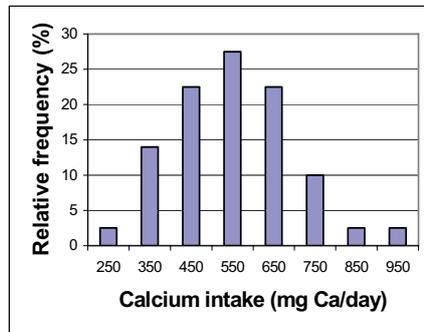


Figure 1: The daily calcium intake of women with mixed diets

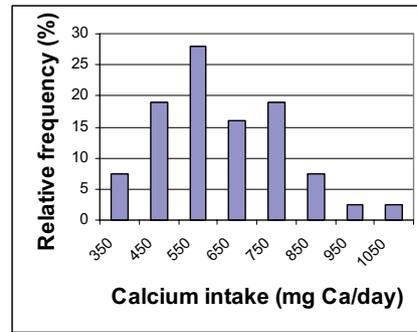


Figure 2: The daily calcium intake of men with mixed diets

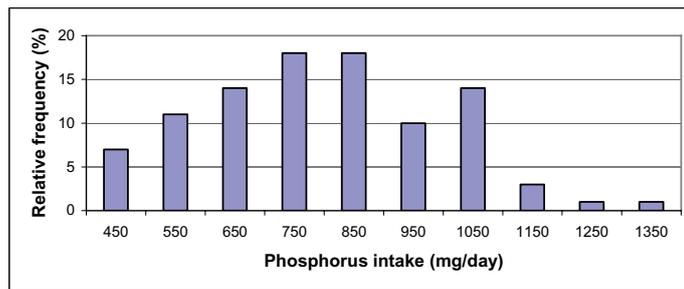


Figure 3: The daily phosphorus intake of women with mixed diets

Die Phosphoraufnahme der Frauen und Männer (Abbildung 3, 4) mit Mischkost bleibt mit 33 bzw. 5 % unter der empfohlenen Aufnahmemenge von 700 mg / Tag, die offenbar zu reichlich postuliert wurde.

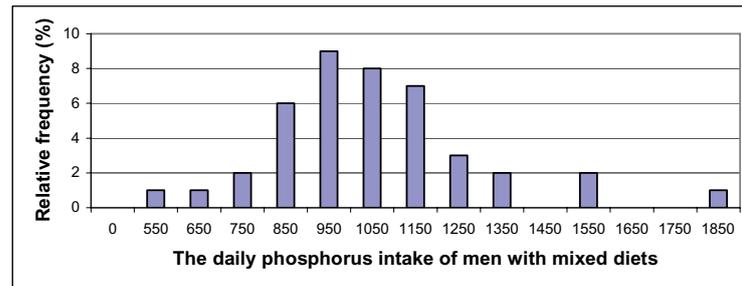


Figure 4: The daily phosphorus intake of men with mixed diets

2. Scheinbare Absorption, Exkretion und Bilanz von Calcium und Phosphor bei Erwachsenen

Das verzehrte Calcium wurde zu 80 % und der konsumierte Phosphor zu 35 % mit dem Stuhl wieder ausgeschieden. Die renale Exkretion des Calcium erreichte 20 %, die des Phosphors 65 % (Tabelle 4). Die scheinbare Absorption des Calcium betrug 0 % bei der Frau und 16 % beim Mann, die des Phosphors erreichte bei beiden Geschlechtern 65 %.

Table 4: Calcium and phosphorus intake, excretion, and apparent absorption rate and balance of women and men with mixed diets

Parameter	Calcium			Phosphorus		
	Women	Men	%	Women	Men	%
Intake	586	785	134	851	1167	137
Faeces, mg/day	594	662	111	385	390	101
Urine, mg/day	135	196	145	599	826	138
Faeces, %	81	77	–	39	32	–
Urine, %	19	23	–	61	68	–
Apparent absorption, %	-1.4	16	–	55	67	–
Balance, mg/day	-143	-73	–	-132	-49	–
Balance, %	-24.4	-23	–	-15.5	-4.2	–

Die Calciumbilanz der Frauen (Tabelle 5) im Alter von 20 bis 69 Jahren ist im Mittel negativ, die des Phosphors (Tabelle 6) auch. Die Frauen und Männer dieser 3 einzeln dargestellten Testpopulationen im Alter von > 40 Jahren verlieren mehr Calcium und Phosphor als sie wieder inkorporieren. Die Calcium- und Phosphorbilanz dieser 3 Populationen ist aus diesem Grund im Mittel negativ.

Diskussion der Befunde

Der Calcium- und Phosphorgehalt pflanzlicher Lebensmittel aus konventionellem Anbau schwankt zwischen ca. 30 mg Ca bzw. 20 mg Phosphor / kg TM im Zucker

und 12000 mg Ca bzw. 125000 mg P / kg TM in Gurken (*Tabelle 8*). In der Regel enthalten die pflanzlichen Lebensmittel zweimal mehr Phosphor als Calcium.

Table 5: Calcium intake, excretion, apparent absorption rate and balance of women and men with mixed diets

Parameter		Women			Men		
		A	B	C	A	B	C
Intake	mg/day	645	547	567	812	765	779
Excretion	Faeces mg/day	768	420	593	855	624	507
	Urine mg/day	154	153	98	130	181	216
	Faeces %	83	73	86	82	78	70
	Urine %	17	27	14	18	22	30
Apparent absorption	%	-19.1	23.2	-4.6	-5.2	18.4	34.9
Balance	mg/day	-277	-26	-124	-233	-40	56
	%	-42.9	-4.8	-219	-28.7	-5.2	7.2

Table 6: Phosphorus intake, excretion, apparent absorption rate and balance of women and men with mixed diets

Parameter		Women			Men		
		A	B	C	A	B	C
Intake	mg/day	966	753	835	1065	1262	117
Excretion	Faeces mg/day	408	333	413	470	365	336
	Urine mg/day	651	573	573	777	924	77
	Faeces %	39	37	42	38	28	30
	Urine %	61	63	58	62	72	70
Apparent absorption	%	58	56	51	56	71	71
Balance	mg/day	-93	-153	-151	-182	-27	60
	%	-9.6	-20	-18	-17.1	-2.1	5.1

Table 7: Calcium balance of young and breast feeding women in a placebo controlled double blind study

Parameter		Young women		Breast feeding women	
		Placebo	Preparation	Placebo	Preparation
Intake	mg/day	718	1228	1137	1562
Excretion	Faeces mg/day	621	1086	841	1193
	Urine mg/day	97	142	91	148
	Milk mg/day	-	-	205	221
	Faeces %	86	88	74	76
	Urine %	14	12	8	10
	Milk %	-	-	18	14
Apparent absorption	%	11	17	15	11
Balance	mg/day	-23	+81	-142	-228
	%	-3.3	+6.2	-14	-17

Der Phosphorgehalt der tierischen Lebensmittel ist gleichermaßen doppelt so hoch wie ihr Calciumanteil. Eigentlich liefern nur Milch und Käse mehr Calcium als Phos-

phor. Der höhere Phosphorgehalt des Schmelzkäses resultiert aus dem Natriumphosphatzusatz zum Schmelzen misslungenen Schnittkäses (Krämer 1993).

Table 8: The calcium and phosphorus content of vegetable and animal foodstuffs in mg per kg dry matter

Vegetable foodstuffs	Ca	P	Animal foodstuffs	Ca	P
Sugar	31	20	Butter	180	210
Starch	200	190	Black pudding	540	1800
Fruits	600	1200	Fish filet	900	2900
Rice, wheat flour	220	1250	Sausage	500	3000
Rolls, rusk	560	1500	Yoghurt	4800	3600
Wheat and rye bread	420	1500	Mutton	450	6000
Potato, sauerkraut	230	3000	Herring	3700	6100
Crisp bread	650	3670	Beef, chicken, pork	240	7250
Oat flakes	660	4300	Rosefish filet	900	8300
Pulses	1000	4600	Trout	2900	8700
Asparagus	2600	4300	Egg	1500	8850
Tomato	3500	8700	Liver, cattle	150	9600
Cauliflower	4500	9500	Milk, cheese	17500	9700
Cucumber	12000	12500	Soft cheese	11500	21000

Mischköstler beider Geschlechter nehmen 68 bzw. 58 % des Calcium und Phosphors über tierische Lebensmittel, 29 bzw. 36 % über pflanzliche Lebensmittel und 3 bzw. 6 % über Getränke auf. Milch und Käse liefern dem Menschen die Majorität des Calcium (Anke 2004). Die Kalkulation der Calcium- und Phosphoraufnahme der Mischköstler nach der Marktkorb- bzw. Basketmethode überschätzt die Calciumaufnahme der Erwachsenen um 44 %, die des Phosphors um 48 % im Vergleich zur chemischen Bestimmung mit Hilfe der Duplikatmethode. Die Verwendung von kalkulierten Calciumverzehrdaten sollte zukünftig unterbleiben. Sie ist auch die Basis für überhöhte Calciumbedarfswerte Erwachsener (Anke *et al.* 2003).

Der empfohlene normative Calciumbedarf von 500 mg/Tag im Wochenmittel für Frauen (60 kg Körpergewicht), 600 mg/Tag für Männer (70 kg Körpergewicht) und 750 mg für Stillende wird den Anforderungen gerecht. Eine Ergänzung mit Calcium ist individuell geboten, eine Verminderung der Phosphorbelastung ist anzustreben.

Summary

The normative calcium requirement of women (50 kg body weight) is 500 mg / day, of breast-feeding women 750 mg / day, and of men 600 mg / day (70 kg body weight). Calcium supplementation of young women with a native calcium intake of 695 mg / day increased their faecal calcium excretion by about the supplemented calcium amount. The calcium balance of young women with an intake of 500 mg Ca / day or 9 mg Ca / kg body weight is equalized. With increasing age (> 40 years), the calcium balance of women becomes more negative than that of men. The apparent absorption rate of men is significantly higher than that of women. Osteoporosis is much more common in women than in men. The phosphorus consumption of 700 mg / day satisfied the phosphorus requirement of women and men.

(Literatur kann beim Autor erfragt werden)

Impact of benzoic acid on early weaned piglets.

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Introduction

The aim of the trial was to study the impact of benzoic acid at the dietary inclusion level of 5 g per kg feed on piglet performance and on composition of the intestinal microbiota. In addition the tolerance of piglets to the feeding of benzoic acid at the 1% level was examined in a wheat based diet judged on pathomorphological examinations, diarrhea incidence and differences in microbial colonization. We applied PCR-DGGE methodology for a comparative examination of the predominant colonic and iliac microbiota, because this molecular technique provided a higher resolution in ecological analysis (Tannock, 1999). Since introduction of this technique by Muyzer et al. in 1993 PCR-DGGE has since been effectively applied to monitor changes in the porcine intestinal and fecal microbiota (Simpson et al. 1999; Collier et al. 2003) due to external perturbation, such as diet and nutrition.

Material and Methods

Fifty four castrated males and females piglets (mothers: German Landrace x Duroc; fathers: Landrace B x Hampshire) were used in equal proportions (50% to 50%) directly after weaning (24th day of life) to the 66th day of life. The piglets were allocated to flat decks of 2 piglets per pen and were allotted equally to the three treatment groups (Control, 0.5%, and 1% benzoic acid) according to the litters and gender. Each treatment consisted of 18 piglets. The room was artificially illuminated for 12 h. The room temperature was constantly 23 °C. The relative humidity was adjusted to 64 ± 3 %.

A typical Starter diet for piglets was used, containing wheat, barley and soybean meal as the main feed ingredients. It was formulated to contain 19% crude protein, 1.20% lysine and 13.4 MJ ME per kg feed. Feed was provided in pelleted form and offered *ad libitum*. Drinking water was continuously supplied. Benzoic acid was added using a premix based on wheat (10%). All diets were analysed for the nutrient contents (dry

matter, crude fiber, crude protein, crude ash, starch, total sugar, calcium, phosphorus, sodium) and benzoic acid concentration.

On the first day of the experiment and at the end of each following week average body weight as well as the amount of feed remaining in the feeder per pen was documented. The weight gain was calculated as the body weight at the end of each period minus the body weight of the respective pen at the beginning of each period. Periodically feed consumption per piglet was calculated as the total amount of feed per pen and period divided by the number of piglets per pen. The feed efficiency of each pen was calculated as quotient of the feed consumption and the total body weight gain.

From day one onwards the appearance of faeces was ranked daily in one of two classes (normal or fluid). Diarrhea was defined as liquid consistency over a minimum of three consecutive days. Diarrhea incidence (%) was calculated as a quotient from the number of newly affected piglets during the first six weeks after weaning and the total number of weaned piglets.

Eight piglets per group, which represented the average body weight of the respective experimental group, were sacrificed at the end of the experiment (day 66 of life) for pathomorphological examinations, analysis of the gut microbiota, investigation of various blood characteristics as well as hippuric acid concentration in and pH of urine. Blood was obtained by venepuncture of the lateral jugularis vene and differential blood cell counts (basophiles, eosinophiles, lymphocytes, monocytes and pseudo-eosinophiles) and standard chemical blood constituents (hemoglobin, hematocrit, potassium, sodium, Chlorides, calcium, total cholesterol, total fatty acids, urea, choline esterase, γ -GT, GOT, GPT, glucose, alkaline phosphatase and total protein) were examined. Subjected to pathological-anatomical examination were the carcass, skin, extremities, body cavities as well as all internal organs with special attention to oesophagus, stomach, intestinal tract, liver, kidneys, lungs, and heart. In the opened stomach changes of the pars oesophagus (hyperkeratosis, erosions, ulcers) were graded according to a modified scoring System. The fundus of each stomach was judged with regard to the presence of areas showing inflammation or mucosal erosions and ulcers. Additionally liver, kidneys and intestine were regularly histopathologically tested.

Colon and ileum digesta of sacrificed piglets were subject to PCR-DGGE analysis and analysis of *E. coli* serogroup isolation frequency. Total DNA was isolated and purified following a modification of previously described methods (Zoetendal et al.

2001). PCR with specific primers for total Eubacteria and *Lactobacillus* spp. amplifying the variable V6 to V8 region of eubacterial 16S rDNA. For subsequent DGGE a linear chemical DNA-denaturing gradient of urea and formamide was formed in polyacrylamide gels. PCR amplicates were loaded in single gel lanes and electrophoresis was performed for 16 h at 120 V. After electrophoresis, gels were silver-stained and developed. Scanned DGGE banding patterns were analyzed and various qualitative and semi-quantitative ecological characteristics known from population statistics (Kwak and Petersen 2001) were determined from the computed data to assess shifts within the predominant microbiota studied (e. g. richness, evenness, diversity and similarity indices). Serogroups of *E. coli* isolates from colon and ileum digesta were analysed and their frequency calculated.

All data were subjected to analyses of variance as completely randomised design. Treatment means were compared using appropriate mean comparison procedures and significance level was set at $P < 0.05$.

Results

The trial was conducted without any major disturbances in animal health. The nutritional values and the benzoic acid were in the intended range.

The average body weight of the control group without benzoic acid increased from 9.8 kg at the beginning of the trial up to 29.8 kg at the end of the 6 week period. The average daily weight gain was accordingly 479.5 g per piglet and day. Feeding benzoic acid with 5 g per kg diet improved the overall performance numerically by a 5% increase in daily gain compared to control piglets. However, the difference was not significant. With inclusion of 10 g benzoic acid per kg diet the daily weight gain was in the range of the control group. The overall feed consumption showed no significant difference between all treatment groups. The overall feed conversion (kg feed per kg weight gain) of 1.36 for piglets without supplementation characterized the high performance level. Feeding 5 g benzoic acid per kg diet resulted in a numerically slightly lower feed conversion by 1.1% compared to control piglets. Equally, the inclusion of 10 g benzoic acid per kg diet resulted in no significant different feed efficiency compared to both other treatment groups. The incidence of diarrhea was significantly reduced for piglets fed 5 g benzoic acid per kg diet, while for piglets fed 10 g benzoic acid per kg diet a slight numerical increase in diarrhea incidence was observed.

Differential blood cell counts, the electrolytes, enzymes and metabolites showed with the exception of urea, which was significantly lower in piglets fed with 5 or 10 g benzoic acid per kg diet, no differences between the experimental groups. All ranges were within the reference values for healthy piglets. The urine pH was reduced with feeding benzoic acid. The degree of acidification was dependant upon the inclusion rate. The hippuric acid concentration in urine was significantly higher in piglets fed with benzoic acid than in control piglets.

The organ weights of liver and kidneys showed no significant differences between the experimental groups. In all groups hyperkeratosis and erosions/ulcers in the pars oesophagea of the stomach were found. Feeding 10 g benzoic acid per kg diet induced a distinct but not significant increase in the seriousness of changes. Feeding 10 g benzoic acid per kg diet led to a hyperkeratosis of the mucous membrane of the distal oesophagus of two piglets and lesions of the mucosa of the fundus occurred numerically more frequently than in the two other treatment groups.

Effects on the studied microbiota depended on treatment group, sampling site and analysis method. Using serotyping of *E. coli* isolates from colon and ileum digesta of sacrificed piglets a highly significant reduction of several serogroups was observed in the intestinal samples of piglets receiving diets supplemented with benzoic acid. PCR-DGGE banding pattern revealed no differences in richness, evenness and diversity of both sampling sites between all treatment groups. But, intragroup similarity values between eubacterial amplicons derived from colonic and iliac digesta showed marked differences between treatment groups indicating separate group specific clusters. However such trends were not observed for the predominant *Lactobacillus* spp. at any sampling site.

Summary

The aim of the trial was to study the effectiveness of benzoic acid at the dietary inclusion level of 5 g per kg on performance of early weaned piglets. In addition the tolerance of piglets to the feeding of benzoic acid at 1% level was examined in a wheat based diet. A total of fifty four castrated males and females piglets (mothers: Landrace x Duroc; fathers: Landrace B x Hampshire) were used from the 24th to the 66th day of life. The piglets were allocated to flat decks of 2 piglets per pen and were allotted equally according to the litters and gender. Each treatment consisted of 18 piglets. For performance data average

body weight gain, feed intake and calculated feed conversion data per pen were used. Incidence of diarrhoea was registered by daily inspection. Additionally blood cell counts and biochemistry parameters as well as post-mortal pathomorphological examination, pH and hippuric acid in urine were evaluated in eight piglets per group. The study demonstrates with feeding 5 g benzoic acid per kg diet a numerically slightly improved body weight gain, a reduced incidence of diarrhoea and a significant higher hippuric acid concentration and a not significantly lower pH in the urine than in piglets without feeding benzoic acid. No negative effects on blood and pathologic-anatomical examination were found. Feeding 10 g benzoic acid per kg diet showed no positive effect on performance. The acidification in the urine was more pronounced than in piglets feeding 5 g benzoic acid per kg diet. Feeding the 1% level induced a distinct but not significant increase in the seriousness of mucosa lesions of the pars oesophagea of the stomach compared to the 0.5% level and the control group. Mucosa lesions in the oesophagus and in the fundus of the stomach were more frequent than in piglets fed with the 0.5% level or fed without benzoic acid. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and *E. coli* isolate serotyping was further applied to assess benzoic acid induced alterations in the predominant colonic and iliac microbiota from weaning pigs. Calculation of various ecological characteristics and *E. coli* serogroup isolation frequency does indicate an modification of local microbial composition in colon and ileum digesta due to dietary benzoic acid supplementation.

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Addition of inulin to diets low or high in NSP does not stimulate bifidobacteria and lactobacilli numbers in the intestine of growing pigs.

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Introduction

Digestion and absorption of feed must be completed prior to the large intestine. In the jejunum and colon, undigested feed will be used as a substrate by the intestinal microbiota which is important for maintenance of gut health. From the time of birth, the intestinal microbiota is in a state of flux (Pluske et al., 2002). The balance between beneficial bacteria (lactobacilli and bifidobacteria) and potential pathogenic bacteria (*Escherichia coli*, Salmonella, Clostridia) is susceptible to external influences or agents. Factors that can modify this balance include antibiotic administration, stress, environmental and management changes and disease challenges (Williams et al., 2001). Commercial farming conditions place various stressors resulting in poor growth performance, increased mortality and digestive disorders. It would appear to be to the advantage of the farm animals to attempt to maintain intestinal health and prevent some of this disorders, which are associated with pathogenic bacteria. Maintenance of a symbiotic relationship between host animal and its intestinal microbiota is now recognized as an advantageous nutritional strategy (Williams et al., 2001). The use of antibiotic growth promoters in farm animals resulting in the development of bacterial resistance to antibiotic substances used in human medicine fuels the search for alternative nutritional feed ingredients (Lebek & Gubelmann, 1979). A total ban on the use of these antibiotic substances in farm animals will be in place in Europe by 2006 (Simon et al., 2004). As an alternative the prebiotic concept to modify the microbiota in association with increasing number of beneficial bacteria is based on the administration of indigestible carbohydrates. In the early 1980s in Japan it was realised that non-digestible carbohydrates are essential part of human food (Van Loo et al., 1999). The beneficial effect on the intestinal health of animals and man was related to the presence of bacterial which are able to ferment oligo- and polysaccharides (Mathers & Annison, 1993). There are many health effects associated with intake of prebiotics (Stewart et al., 1993), many of which have not been proven and in many cases, the mechanisms of these effects are unknown.

More recent research has confirmed that dietary oligosaccharides, and in particular, fructo-oligosaccharides (FOS) stimulate bifidobacteria and lactobacilli in number and activity and depress pathogenic bacteria such as *E. coli* or clostridia in man (Kleessen et al., 1997). Oligosaccharides have now become a normal ingredient in weaner feeds for piglets in Japan. It is now well-established in man that oligosaccharides like inulin reach the large intestine and are fermented by the colonic microbiota (Rasmussen et al., 1988) This is associated to the production of short chain fatty acids, hydrogen, carbon dioxide and biomass (Bugaut & Bentéjac, 1993). Recent studies indicated that intestinal colonization in pig differs from that of man and the bacterial break-down of prebiotic, such as inulin, occurs largely in the small intestine (Branner et al., 2004). The purpose of the present study was to investigate whether inulin added to high or low cereal based diets has a bifidogenic influence and an impact on selected bacteria strains in the small and large intestine in growing pigs. The microbiota of man and animal is diverse and contains between 200 and 400 different microbial strains (Holdeman et al., 1977). Due to the complex nutritional requirements, about 60 % of the bacterial population can not be cultivated (Tannock et al., 2000). Thus, the microbiota has been analysed by using fluorescence-*in-situ*-hybridization (FISH) and gut wall-associated population of lactobacilli by denaturing gradient gel electrophoresis (DGGE).

Materials and Methods

After weaning (28 days of age) and run-in period (2 weeks), 8 male castrated piglets (German Landrace) per treatment with a mean body weight of 9.2 (SD 1.4) kg were fed (1) a high cereal fibre (NSP) diet (HF), (2) a high cereal fibre diet+inulin (HF+I), (3) a low cereal fibre (NSP) diet (LF) or (4) a low cereal fibre diet+inulin (LF+I, table 1). The diets were composed to be isonitrogenous and isoenergetic. Inulin was added at a level of 30 g·kg⁻¹ feed (Inulin, Raftiline® ST, Orafiti, Tienen, Belgium) to HF+I and LF+I. The inulin contained 8 % of glucose and fructose and the average degree of polymerisation (DP) was 12. Piglets were housed individually in metabolism cages and were fed twice per day at a level of 80 g food per kg^{0.75} bodyweight. After 21 and 42 days of feeding the test diets, the animals were slaughtered by exsanguination. The total GIT was removed and intestinal contents and tissue samples of about 2 cm length of stomach, jejunum and colon were

collected. Fluorescence-*in-situ*-hybridization with 16S/23S rRNA-targeted oligonucleotid probes was applied to quantify total bacteria (a mix of five probes: Bact 338, Bact 785, Bact 927, Bact 1055 and Bact 1088; Amann et al., 1995), bifidobacteria (S-G-Bif-0164-a-A-18, Langendijk et al., 1995), lactobacilli (S-Lacb-0772-a-A-25, Sghir et al., 1998), *Escherichia coli* (Poulsen et al. 1995) and clostridia (S-Chis-0150-a-A-23 and S-Clit-0135-a-A-19, Franks et al., 1998) in chyme using an epifluorescence microscope. DGGE analysis was applied for detection of strains of lactobacilli by specific primers (Lab-0159-a-S-20 and Univ-0515-a-A-24, Heilig, 2002) using PCR-based techniques in bacterial DNA extracted from gut wall samples. In jejunal and colonic chyme samples, we investigate the concentration of inulin according to the AOAC method 997.08.

Table 1. Chemical composition of the four test diets

Analysed composition (% of DM)	HF	HF+I	LF	LF+I
Dry matter DM (%)	88.6	88.7	90.4	90.6
Ash	6.1	5.7	6.2	6.3
Crude protein	19.2	19.4	21.5	21.8
Crude fat	4.0	3.9	4.6	4.6
Sugar ²	6.5	9.6	4.8	8.5
Total pentosans	4.1	4.1	3.2	3.2
Soluble pentosans	0.6	0.6	0.1	0.1
β-Glucans	1.8	1.8	0.1	0.1
Starch	48.0	48.6	51.1	51.1
NSP	16.2	15.8	11.8	10.7
ME MJ·kg ⁻¹ DM	14.5	14.4	14.8	14.7

¹Premix, content per kg DM: 6.5% P, 23.0% Ca, 5.0% Na, 20,000 I.U. vitamin A, 2,000 I.U. vitamin D₃, 60 mg vitamin E, 200 mg Cu

²Sugar: water-soluble fraction of di- and monosaccharides

HF/HF+I – High fibre diet ± Inulin

LF/LF+I – Low fibre diet ± Inulin

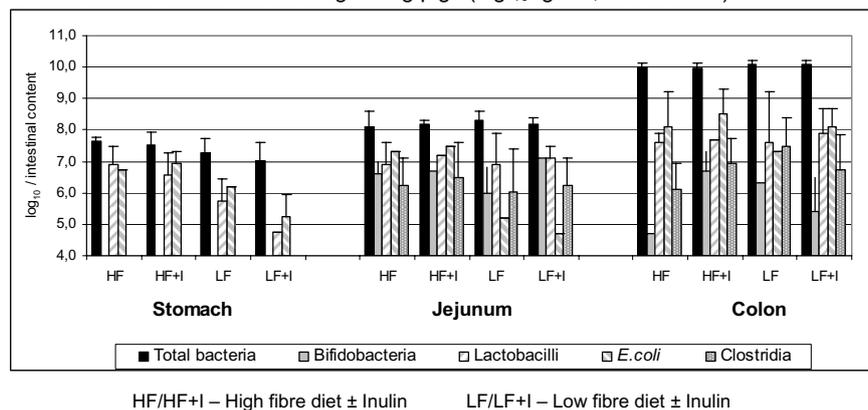
NSP – Non-starch polysaccharides

Results

Piglets were generally in good health and no cases of diarrhoea were found. Piglets receiving HF or HF+I gained 344±36 g and LF or LF+I gained 320±33 g in daily weight gain, respectively, during this trial. No significant effect of inulin addition on body weight gain were observed. Bacteria were present in the stomach, caecum and colon (Figure 1). The feeding period was not a significant factor. Thus, for statistical

calculation data were pooled from both feeding periods. Total bacterial counts ranged from 7,0 (stomach) – 10,0 (colon) \log_{10} / g chyme (wet weight). In stomach, bifidobacteria and strains of clostridia were not detected. No significant differences between diets were observed for the total counts and counts of lactobacilli and coliforms in stomach. A significant effect of inulin on bifidobacteria and lactobacilli in jejunum or colon was not found. In contrast to other studies, our bifidobacteria counts were relatively low and the recovery of bifidobacteria from intestinal chyme ranged from 0 to 100 % with bifidobacteria observed in 15 (jejunum) and 6 (colon) piglets out of 32 piglets.

Figure 1. Total bacteria counts and counts of bifidobacteria, lactobacilli, *E. coli* and clostridia in intestinal contents in growing pigs (\log_{10} /g ww; means \pm sd)



The community of lactobacilli associated with the mucosa surface was analysed by PCR amplification of a fragment of 16S rRNA genes and DGGE. DGGE was performed in tissue samples from two individuals of each diet group. The DGGE pattern reflect predominant lactobacilli attached to the epithelial surfaces of stomach, jejunum and colon. According to the numbers of bands per lane, we found no significant effect of inulin or NSP on lactobacilli community. On average 14 lactobacilli strains per animal and diet was found. The bacterial fermentation of inulin in jejunum amounted to 20 – 40 % and in colonic samples, inulin was completely degraded.

Summary

The problem of post-weaning diarrhoea and the ban of antibiotic growth promoters in piglets requires the changes in the animal diet. Stimulation of a host-beneficial microbiota can be achieved by specific indigestible carbohydrates. The present study examined the microbiota of the gut in piglets, with emphasis on the population of bifidobacteria and lactobacilli. An attempt was made to clarify the influence of inulin in relation to certain bacteria strains with molecular methods. Our results demonstrate that the dietary impact of inulin on the microbiota in pigs is low and the composition of the intestinal microbiota remains remarkably stable after weaning. Unlike man it was shown that inulin is fermented up to 40 % by intestinal bacteria in jejunum of pigs. Finally the molecular methods used in this work are feasible tools to measure alteration in the bacterial gut composition.

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Effects of a probiotic *Lactobacillus acidophilus* strain on food tolerance in dogs with non-specific dietary hypersensitivity

(Effekte eines probiotischen *Lactobacillus acidophilus* Stammes auf die Futtermitteltoleranz von Hunden mit unspezifischer Futtermittelsensitivität)

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The objective of this study was to investigate the effects of a probiotic *L. acidophilus* strain DSM 13241 on food tolerance in dogs with non-specific dietary sensitivity.

6 adult German Shorthair Pointers (4 males, 2 females, body weight 30.8±2.0 kg) with a history of non-specific dietary sensitivity were fed a control dry diet, the same diet with 6 x 10⁶ colony forming units (CfU)/g *L. acidophilus* DSM 13241 for 12 weeks each and the control diet for additional 4 weeks. Frequency of defecations, scoring of fecal quality, fecal dry matter and fecal unbound water were determined. Fecal samples were cultured for *Clostridium perfringens*, *Escherichia coli*, *Lactobacillus spp.* and *Bifidobacterium spp.* *In situ* hybridization was performed in fecal samples for *Clostridium histolyticum*, *E. coli*, lactobacilli and bifidobacteria. The digestibility of test and control diets was also assessed. Statistics were performed by ANOVA and Student's t-Test, p<0.05.

Feeding *L. acidophilus* DSM 13241 led to a slightly lower frequency of defecations, slightly improved fecal consistency and increased fecal dry matter (p<0.05). It resulted in higher fecal numbers of *Lactobacillus spp.* and *Bifidobacterium spp.* and in a slightly decreased number of *C. perfringens* and *E. coli* as measured by selective bacterial culture. Fluorescence *in situ* hybridization showed less variations in the bacterial concentrations. Digestibility of crude protein and crude fat was higher during the probiotic intake.

This study showed that *L. acidophilus* DSM 13241 can stabilize the digestive processes in dogs with non-specific dietary sensitivity. The observed improvement in fecal consistency would have considerable practical importance, since *L. acidophilus* probiotics are generally regarded as safe (GRAS status) and as such are acceptable for long-term application.

Influence of different pre-, pro-, and synbiotics on the digestibility processes in pigs.

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Introduction

The bacterial community resident in the intestinal tract of human and animals has a major impact on the gastrointestinal function and thereby on the host's health and well-being. Therefore, it is necessary to maintain the balance of the bacterial flora in order to assure the optimal function of the intestine. Considerable efforts have been made to search for biological preparations with a preventive function of protecting the host from various types of intestinal diseases, and gaining positive effects on digestive processes and stimulating influence on the growth of organism. Pre-, pro-, and either synbiotics are food products that are specially designed for this purpose (Simmering and Blaut, 2001), whereas prebiotics are carbohydrates selectively metabolized by desirable moieties of the indigenous flora, probiotics involve the use of live microorganisms in food and synbiotics combine these two approaches. The pre-, pro-, and synbiotic concept was primarily used in human nutrition, but in recent years there has been a growing interest to use these food additives in animal nutrition. Especially in pig nutrition additives are important for the stabilization of the intestinal flora as an alternative for antibiotic growth promoters. Therefore, the aim of the present study was to investigate the influence of different pre-, pro-, and synbiotics on the digestibility processes in pigs. This purpose includes the determination of the precaecal and faecal digestibility of prebiotics and prebiotics combined with probiotics, in order to obtain information about their reactions in the gastrointestinal tract of pigs. Furthermore, the effects of these additives on nutrient digestibility and microbial characteristics in chyme and faeces were examined.

Material and Methods

Animals: Four metabolic trials were carried out with a total of 22 growing male castrated pigs (German Landrace x Pietrain). Eleven pigs were prepared with an ileo-rectal anastomosis (IRA) in end-to-end technique with preserved ileo-caeco-colic valve (Roth-Maier et al., 1998). The other eleven pigs were used as partners for the

surgically fitted animals, so that each pig with IRA had an intact partner (IN). All pigs were treated similarly.

Experimental design and feeding: The experiments were applied in a Latin square design. Each animal received each diet during the experimental periods, whereas one period lasted over 12 days. An exception displayed trial 2 with experimental periods of 40 days in order to investigate if a prolonged adaptation time enhance the possible effects on the microbial characteristics. Treatment effects were examined by two-way analysis of variance. The classification factors were supplementation and procedure. The pigs received a basic diet, based on corn (32 %), wheat (19.6 %), barley (6.5 %), soybean meal (19.4 %), and vegetable oil (2.8 %), which was formulated to provide 13.4 MJ ME and 150 g CP per kg feed. The diet was created to meet the nutrient requirement of swine according to GfE (1987) and NRC (1998). The basic diet was used as control diet (CON) in each trial and was supplemented with the following pre-, pro-, and synbiotics feed additives (amounts of the substances were in accordance with producer's recommendations):

Trial 1: 3 % lactulose sirup (LAC I; responding to 1.5 % pure lactulose) and 2 % long-chain inulin (INU I; DP \geq 23)

Trial 2: 4 % lactulose sirup (LAC II; responding to 2.0 % pure lactulose).

Trial 3: 2 % middle-chain inulin (INU II; DP 10–12), 8×10^9 CFU *Enterococcus faecium*/kg feed (*E.f.* I; DSM 10663) and a synbiotic combination of INU II + *E.f.* I

Trial 4: 0.3 % Mannanligosaccharides (MOS), 5×10^8 CFU *Enterococcus faecium*/kg feed (*E.f.* II; DSM 7134) and a synbiotic combination of MOS + *E.f.* II.

The synbiotic combinations contained the same dosage as used in the single applications. The daily dry matter intake was about 1350 g in trial 1, 2 and 3, and 1650 g DM/d in trial 4. The feed was offered twice daily as a slurry (feed:water 1:2). Additionally, every pig was provided with 150 ml electrolyte solution (composition in g/1000 ml: 3.218 g Na, 0.195 g K, 0.036 g Mg, 0.1 g Ca) according to Hennig et al. (1989). Water was offered *ad libitum* within one hour after feeding.

Surgical procedure: For the present investigations the IRA technique was used as surgery method in order to avoid colonic digestion. Additionally, this method offers the advantage to provide a mixture of the chyme of the whole digestive tract. The principle of fitting an IRA in end-to-end technique is to completely separate the

caecum from the colon. These segments of the gut were entirely disconnected from the functional gastrointestinal tract. The distal section of the ileum, including the ileo-caecal valve, and the proximal section of the rectum were joined end-to-end by individual sutures. The animals had a remaining rectum of about 15 cm minimum and the post-surgical excretion of the chyme proceeds the normal way via the anal sphincter. To avoid caeco-colic tympany a percutaneous plastic canula was implanted as a permanent caecostoma in the left abdominal wall (Roth-Maier et al., 1998).

Metabolic trials and samples collection: The experimental periods were performed in weeks 3 - 21 after surgery. In this period, it is proven that alterations in the microbial population of the ileum can be excluded (Roth-Maier et al., 1998; Hennig et al., 2004). For the quantitative digesta collection the animals were housed in metabolic cages. The diets containing the test substances were offered twice a day. Precaecal effluent, expelled via the anus, and faeces were collected separately from urine for the final 5 days of each period. After collection, digesta were immediately weighed and stored at -20°C. On the last day of the collection period fresh samples of the digesta were taken directly from the anus for the determination of the carbohydrate concentration, bacterial population, pH, short chain fatty acids (SCFA), lactic acid (LA), and ammonia (NH₃).

Analysis and microbiological determination: In feed, freeze-dried chyme, and faeces dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), and crude fibre (CF) were determined (Naumann and Bassler, 1988). Precaecal and total tract nutrient digestibilities were calculated as the ratio of the differences between nutrient intakes and nutrients recovered in the digesta, to the nutrient intake. The amount of lactulose and inulin in feed and digesta was assessed according to Quigley et al. (1998). Bacterial counts were detected using selective medias [Gram-positive anaerobes (Columbia-CNA-Agar), Gram-negative anaerobes (Schaedler-Kanamycin-Agar), lactobacilli (MRS-Agar), coliforms (MacConkey-Agar), enterococci (Slanetz-Bartley-Agar)]. The concentration of SCFA and LA in chyme and faeces was determined by HPLC technique and NH₃ with a commercial available testkit (Randox, Germany).

Results

The precaecal digestibilities of the used pre- and synbiotics are shown in Table 1.

Table 1: Precaecal digestibility of different pre- and synbiotics in growing pigs

Supplementation	LAC I	LAC II	INU I	INU II	INU II + <i>E.f. I</i>
Prebiotic intake (g/d)	22.5 ± 0	30.2 ± 0	30.0 ± 0	30.0 ± 0	30.0 ± 0
Chyme prebiotic excretion (g/d)	4.6 ± 1.1	4.8 ± 1.1	0.7 ± 0.5	12.8 ± 7.8	13.4 ± 4.3
Precaecal digestibility (%)	79.4 ± 4.8	76.9 ± 5.4	97.5 ± 1.8	57.3 ± 26.0	55.5 ± 14.5

The faecal digestibility of LAC I, LAC II, INU I, INU II, and INU II + *E.f. I* was determined as 100 %. An extended application period of lactulose in trial 2 (LAC II) did not alter the precaecal digestibility. Surprisingly, the long chain inulin (INU I) was degraded more precaecally compared to the middle chain inulin (INU II). The determination of MOS in digesta was not possible, because evaluation of an analytical method is still not finished.

Furthermore, the present study showed, that in general the digestibility of nutrients was not affected by the supplementation of the used additives. An exception was the digestibility of DM during the supplementation of lactulose and INU I and the digestibility of CF, when INU II was fed. The addition of lactulose in trial 1 (LAC I) slightly enhanced the precaecal DM digestibility from 75 % (CON) to 77 % (LAC I) and the faecal DM digestibility from 85 % (CON) to 87 % (LAC I). The addition of lactulose in trial 2 (LAC II) increased the faecal digestibility of DM from 81 % (CON) to 84 % (LAC II) significantly. INU I enhanced the faecal DM digestibility from 85 % (CON) to 87 % (INU I). INU II heightens the precaecal CF digestibility from 14 % (CON) to 20 % (INU II) and 24 % (INU II + *E.f. I*) significantly. As a result of these findings, lactulose, INU I and INU II affected the daily nutrient excretion. LAC I and INU I significantly influenced the daily DM excretion, whereas it was reduced in IRA pigs from 340 g/d (CON) to 307 g/d (LAC I) and 318 g/d (INU I) and in IN pigs from 207 g/d (CON) to 176 g/d (LAC I) and 171 g/d (INU I). With the supplementation of lactulose in trial 2 (LAC II) the daily DM excretion of IN pigs was significantly reduced from 267 g/d (CON) to 219 g/d (LAC II). The addition of INU II decreased the precaecal excretion of CF from 42 g/d (CON) to 39 g/d (INU II) and 37 g/d (INU II + *E.f. I*) significantly. The microbial analysis revealed that the factor supplementation had no significant influence on the composition of the microflora. Nevertheless,

various tendencies were observed (Table 2). The different pre-, pro-, and synbiotics had little influence on SCFA-, LA- and NH₃-concentration and on the pH value.

Table 2: Effects of supplementation of pre-, pro-, and synbiotics on bacterial counts in digesta of growing pigs with or without IRA^{1,2,3}

	IRA	IN	IRA	IN	IRA	IN	IRA	IN
Trial 1	<i>control</i>		<i>lactulose I</i>		<i>inulin I</i>			
Gram-negative anaerobes	8.4 ^a ± 0.8	6.8 ^{ab} ± 0.5	7.1 ^{ab} ± 0.9	6.9 ^{ab} ± 0.1	8.5 ^a ± 0.2	6.4 ^b ± 0.6		
Enterococci	7.7 ^{ab} ± 0.2	7.0 ^{ab} ± 0.7	7.8 ^{ab} ± 0.5	6.4 ^{ab} ± 1.2	8.2 ^a ± 0.1	6.1 ^b ± 1.0		
Coliforms	6.9 ± 0.5	6.8 ± 1.7	7.7 ± 1.1	7.5 ± 1.1	7.2 ± 1.4	8.0 ± 1.1		
Trial 2	<i>control</i>		<i>lactulose II</i>					
Gram-positive anaerobes	9.2 ^a ± 0.4	8.7 ^{ab} ± 0.3	9.0 ^{ab} ± 0.4	8.2 ^b ± 0.5				
Enterococci	8.8 ^a ± 0.5	7.9 ^b ± 0.6	8.6 ^a ± 0.3	7.4 ^b ± 0.4				
Coliforms	8.3 ± 1.0	7.1 ± 0.6	6.9 ± 1.3	7.5 ± 0.7				
Trial 3	<i>control</i>		<i>E. faecium I</i>		<i>inulin II</i>		<i>inulin II / E.f. I</i>	
Gram-negative anaerobes	6.2 ^{ab} ± 0.8	4.0 ^b ± 0.5	7.5 ^a ± 1.4	4.0 ^b ± 0.9	6.3 ^{ab} ± 1.2	5.0 ^{ab} ± 2.2	6.5 ^{ab} ± 0.8	4.0 ^b ± 0.7
Enterococci	7.0 ^{abc} ± 1.4	6.5 ^{abc} ± 0.6	7.8 ^{ab} ± 0.9	6.3 ^{ac} ± 0.2	8.0 ^{ab} ± 1.1	5.9 ^c ± 0.6	8.2 ^a ± 0.7	6.9 ^{abc} ± 1.0

¹⁾ Data are means ± SD. ²⁾ Bacterial counts: ¹⁰log colony forming units per g fresh digesta. ³⁾ Means with different superscripts differ significantly ($P < 0.05$).

Discussion

The present investigations showed that the used prebiotics are digested and absorbed by pigs to a considerable degree in the ileum. The small amount of prebiotics, resisting digestion in the ileum, was completely degraded in the colon. The additional supplementation of probiotics, used to obtain a synbiotic combination, reduced the precaecal digestibility of prebiotics marginally. The high disappearance rate of prebiotics in the small intestine of pigs may be due to the high number of bacteria in that part of the intestinal tract (Conway, 1994). The present results concerning the precaecal digestibility of prebiotic carbohydrates are contrary to results from human research, which report high precaecal recovery rates of prebiotics in man (Bach Knudsen and Hesso, 1995).

Furthermore, the other investigated parameters (nutrient digestibility, bacterial counts, pH, SCFA, LA, NH₃) were only slightly influenced by the supplementation of

the pre-, pro-, and synbiotic feed additives. The intention to scientifically prove the positive effects of pre-, pro-, and synbiotic carbohydrate in pigs was only partly successful. It is questionable whether this prebiotic concept can achieve any greater additional beneficial effects, because pigs usually receive already high amounts of prebiotic components with their feed.

Summary

Four digestibility trials were carried out with a total of 22 male castrated pigs. Half of the pigs were fitted with an end-to-end ileo-rectal anastomosis with preserved ileo-caeco-colic valve; the remaining pigs were used as intact partners. The animals received a basal diet supplemented with either pre-, pro-, and synbiotic feed additives (per kg): lactulose (1.5 and 2%), inulin (long-chain (I) and middle-chain (II); 2%, resp.), *Enterococcus faecium* (DSM 10663, 8×10^9 CFU/kg and DSM 7134, 5×10^8 CFU/kg), Mannanooligosaccharides (0.3%) and the synbiotic combination inulin II + *Enterococcus faecium* (DSM 10663) and Mannanooligosaccharides + *Enterococcus faecium* (DSM 7134). The prebiotics were mainly digested precaecally and completely faecally. Nutrient digestibility, bacterial counts, pH value, short chain fatty acids, lactic acid, and ammonia were widely unaffected by the added pre-, pro-, and synbiotics. Because of high amounts of prebiotic substances in the usual pig feed their effectiveness in pig nutrition is questionable.

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Modifying gut flora in pigs by phytochemicals

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Introduction

Regulation 1831/2003 (EC) lays down provisions phasing out the authorisations of antimicrobial growth promoters (AMGP's) in the EU as from January 2006. The antimicrobial properties of certain plant-derived chemicals (phytochemicals) have been described extensively, mostly based on observations from semi-quantitative diffusion methods or microtiter plates, in non-gut conditions (e.g. Cowan, 1999). However, there is little quantitative data on their antimicrobial activity against the normal gut flora of the pig. These phytochemicals have potential as an alternative for the AMGP's if their mode of action is based on effects on the gut flora and/or the animal's digestive functions. In this research, the *in vitro* antimicrobial activity of 7 phytochemicals (monoterpenes; carvacrol, thymol, eucalyptol and terpinen-4-ol; phenylpropenes; trans-anethole, eugenol and cinnamaldehyde) against the main components of the pig gut flora has been evaluated. Furthermore, it is interesting to know whether these chemicals are degraded in the gut.

Material and Methods

***In vitro* incubation.** The *in vitro* incubation medium was composed of 2 g of an artificial substrate (carbohydrate, protein, fat, mucine, vitamins, minerals, antioxidants and bile acids (only small intestine)), 20 ml of a buffer solution (pH 3, 6.5 and 6.5 for simulation of gastric, small intestinal and caecal fermentation respectively), 1 ml of a suspension of pig gut flora (inoculum) and 50 µl of a solution of the phytochemical in absolute ethanol. The duration of the *in vitro* incubation was 3, 4 and 24h for simulation of gastric, small intestinal and caecal fermentation respectively, allowing an exponential growth of the bacteria. A control (0 ppm in medium) and three concentrations were tested per phytochemical. Bacterial counts (viable counts; CFU log₁₀/ml) on starting media and after incubation were done using the ring-plate technique of Van Der Heyde & Henderickx (1963). Selective media were used for counting the

following groups: total anaerobic bacteria, *Escherichia coli*, streptococci and lactobacilli. All incubations were done in triplicate. Statistical analysis was performed per phytochemical and per type of simulation by the GLM ANOVA procedure with the fixed effect of concentration and replicate as a random factor to account for variation in the inoculum. Means for effects of concentration were compared using Duncan's multiple range test ($P < 0.05$).

GC-method to quantify phytochemicals using an internal standard. Samples taken before and after *in vitro* incubation were acidified to pH 2 with 2% of 24 M H_2SO_4 to stop fermentation. Each quantification was performed in duplicate. The sample size for extraction was 2 ml. Each sample was spiked with 100 μ l of 2-isopropyl-phenol internal standard solution and extracted twice by vigorous shaking for 2 min with 4 ml of ethyl acetate. The samples were then centrifuged (1100 g, 3 min) prior to transfer of the less dense ethyl acetate layer. The combined ethyl acetate extracts were reduced to dryness with nitrogen at room temperature. The residue of each was redissolved in ethyl acetate (500 μ l). Aliquots (1 μ l) were used for GC-analysis. GC was performed using a capillary column 30 m x 0.25 mm ID x 0.25 μ m film thickness fused silica. The phase composition was 2,3-di-O-methyl-6-O-tert-butyl dimethyl silyl beta cyclodextrin doped into 14% cyanopropylphenyl / 86% dimethyl polysiloxane (Restek Rt- β DEXsm; chiral separation column). The method has been validated for linearity, specificity, recovery of extraction/drying and repeatability. Data on concentration of phytochemicals before and after incubation were expressed as % recovery (concentration of phytochemical after incubation / concentration of phytochemical before incubation * 100%). These recoveries are a measure for degradation. The mean % recovery of three replicates were compared with a test value of 100% by means of an one-sample T-test.

Results and discussion

Stomach. The natural barrier of the stomach for bacterial growth was reinforced by carvacrol and thymol at elevated concentrations. Thymol had the highest antibacterial activity and showed significant lower numbers for total bacteria and *E. coli* at 100 and 500 ppm and for streptococci and lactobacilli at 500 ppm (Figure 1). Carvacrol significantly lowered all bacterial counts at 500 ppm compared to control but not at 100 ppm. Remarkably, cinnamaldehyde had no effect on streptococci or lactobacilli but reduced the number of *E. coli* from 5.8 to 4.5 at 500 ppm ($P < 0.05$). Eucalyptol, ter-

pinen-4-ol, trans-anethole and eugenol had no significant effects.

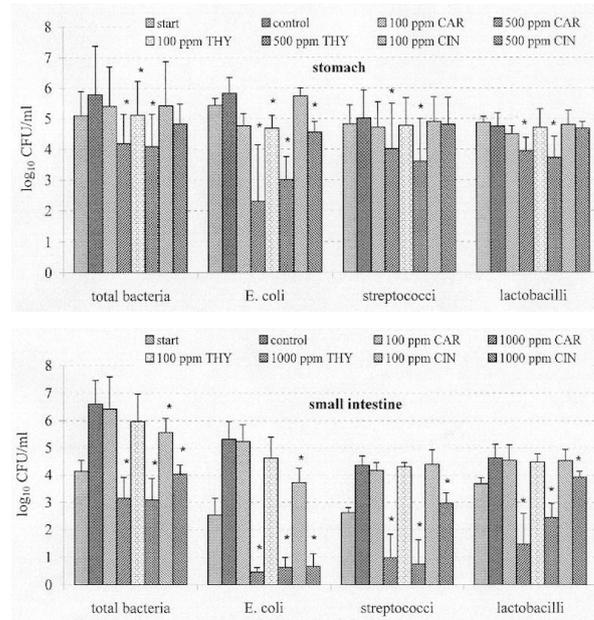


Figure 1. *In vitro* antimicrobial properties of carvacrol (CAR), thymol (THY) and cinnamaldehyde (CIN) on pig gut flora (* = sign. different from control, $P < 0.05$)

Small intestine. The *in vitro* incubations revealed that carvacrol and thymol had very strong antimicrobial activity at 1000 ppm, but no significant differences in bacterial counts were found at 100 ppm (Figure 1). A very low number of *E. coli* was still present after incubation, 0.5 and 0.6 CFU \log_{10} for carvacrol and thymol respectively. Cinnamaldehyde significantly diminished total bacteria and *E. coli* at 100 and 1000 ppm. In contrast to carvacrol and thymol, cinnamaldehyde did not reduce the G+ streptococci and lactobacilli so dramatically, which is in line with the results for the gastric incubations. Besides the treatment of 100 ppm (no significant effects), the results for eugenol were comparable to those of cinnamaldehyde (Table 1). Both phytochemicals seemed to have a more selective antimicrobial spectrum. Terpinen-4-ol at 1000 ppm gave a significantly lower number of total bacteria (- 0.8 CFU \log_{10}) and *E. coli* (- 0.7 CFU \log_{10}). Only total bacteria were significantly reduced by trans-anethole (Table 1). The general higher antimicrobial activity of the phytochemicals found in small intestine compared to that in the stomach and caecum could be

explained by the presence of bile acids in the artificial substrate of small intestine. Ultee *et al.* (1999) illustrated the membrane-interacting mode of action of carvacrol on *Bacillus cereus* and Gill & Holley (2004) hypothesized that the mechanisms of inhibition of energy generation of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei* could be due to the inhibition of glucose uptake or utilization and effects on membrane permeability. The solubility in prokaryotic membranes of lipophilic components (like the phytochemicals used in this research) could be enhanced by the emulsifying activity of bile acids.

Caecum. Caecum conditions are different from those in the other parts of the gastrointestinal tract by the anaerobic conditions and the longer retention time. The results for carvacrol, thymol and cinnamaldehyde were more or less identical. The 1000 ppm treatment gave significant reductions compared to the control for all groups of bacteria (average CFU log₁₀/ml for the 3 phytochemicals were 7.0 vs 9.4 for total bacteria; 6.7 vs 9.1 for *E. coli*; 5.9 vs 8.8 for streptococci and 4.2 vs 8.1 for lactobacilli). 100 ppm was not effective. Piva *et al.* (2002) found a 24% reduction of *Enterobacteriaceae* (4.1 vs 5.4 CFU log₁₀/ml) after 24h of an *in vitro* caecal fermentation with 400 ppm carvacrol. Generally, lactobacilli were more affected than the other groups, which is illustrated by trans-anethole (Table 1) and terpinen-4-ol (7.6 vs 8.5 CFU log₁₀/ml). These phytochemicals had only a significant effect on lactobacilli. Carvacrol and thymol had clearly stronger antimicrobial properties than the latter two monoterpenes, as was also demonstrated by Dorman & Deans (2000). They found that, amongst a high number of terpenes, thymol, followed by carvacrol had the highest and widest spectrum of activity against bacterial growth. In caecal conditions, eugenol reduced only *E. coli* (Table 1).

Table 1. *In vitro* antimicrobial activity of eugenol 1000 ppm (EUG) and trans-anethole 1000 ppm (ANE) on pig gut flora

	small intestinal simulation ⁽¹⁾				caecal simulation ⁽²⁾			
	total bact.	<i>E. coli</i>	Strep-toc.	Lacto-bac.	total bact.	<i>E. coli</i>	Strep-toc.	Lacto-bac.
control	6.6	5.3	4.4	4.6	9.7	9.3	9.1	8.5
EUG	4.2 (*)	1.0 (*)	3.1 (*)	3.8 (*)	9.6	7.5 (*)	9.0	8.3
control	6.2	4.9	4.2	4.6	9.7	9.3	9.1	8.5
ANE	5.5 (*)	4.3	3.9	4.4	9.6	8.5	8.8	7.8 (*)

(* = sign. different from control, P < 0.05); ⁽¹⁾ after 4 h of incubation; ⁽²⁾ after 24 h of incubation

***In vitro* intestinal degradation of phytochemicals.** Samples taken before and after *in vitro* incubation were quantified for their content in cinnamaldehyde, eugenol, thymol and carvacrol and expressed as % recovery. The recoveries for stomach incubation were not significantly different from 100%, so it can be concluded that in gastric conditions no degradation has taken place. Table 2 summarizes the results for small intestine and caecum. Significant degradation in the small intestine could be found for cinnamaldehyde and eugenol at 100 ppm and for cinnamaldehyde at 1000 ppm. This degradation for cinnamaldehyde at 100 ppm accounted for 26.8%.

Table 2. Phytochemical recovery from *in vitro* incubations (conc. of phytochemical after incubation / conc. of phytochemical before incubation * 100%)

	100 ppm		1000 ppm	
	Recovery (%)	Significance ⁽¹⁾	Recovery (%)	Significance ⁽¹⁾
SMALL INTESTINE				
cinnamaldehyde	73.2	**	88.9	*
eugenol	92.1	**	99.9	NS
thymol	107.3	NS	105.8	NS
carvacrol	106.1	NS	107.9	NS
CAECUM				
cinnamaldehyde	(2)		(2)	
eugenol	78.4	*	75.5	*
thymol	77.2	*	83.1	*
carvacrol	71.0	*	89.0	NS

⁽¹⁾ ** = significantly different from 100% at 1% confidence level, * = significantly different from 100% at 5% confidence level, NS = not significantly different from 100%; ⁽²⁾ see text

The GC-method to quantify cinnamaldehyde in caecal contents could not be validated due to a lack of linear peak signal response and unreasonable low response factors of the analyte. It is suggested that a certain amount of this chemical is rapidly (partially) degraded once added to the standard caecum matrix and consequently not detected or interference/adsorption with other products of the caecum matrix had taking place. The exact reason for this analytical problem is not yet fully understood. However, there was no detectable response (< 5 ppm) for cinnamaldehyde for the 100 ppm objects after caecal incubation, which could indicate that this phytochemical was (totally) transformed under caecal conditions. The 1000 ppm object after incubation gave a moderate response but could not be quantified. Cinnamaldehyde has been shown to be readily biodegradable. It was 100% biodegraded after 21 days in an OECD 301B test (Haarmann & Reimer, 2001). Significant degradation for eugenol,

thymol and carvacrol in caecum could be found and varied between 16.9% and 29.0%. The recovery for the 1000 ppm treatment for carvacrol and thymol was 89.0 and 83.1% respectively which is in the same magnitude order of the results of Varel (2002) who found that high doses of carvacrol and thymol (1000-2500 ppm) were stable in swine waste under anaerobic conditions for 62 days, with 90 to 95% of the additive being recovered.

Summary

The *in vitro* antimicrobial activity of 7 phytochemicals against the major, but not all, culturable components of the pig gut flora has been tested. Carvacrol, cinnamaldehyde, thymol and eugenol give opportunities to modulate the flora of the gastrointestinal tract of pigs. The use of cinnamaldehyde and eugenol could result in a higher lactobacilli/*E. coli* ratio in the gastrointestinal tract, while carvacrol and thymol seemed to be less selective. The monoterpenes carvacrol and thymol were stable in gastric and small intestinal conditions, but were degraded up to 29% in caecum. A moderate recovery for eugenol was found in caecum and for cinnamaldehyde in small intestine. No detectable responses (< 5 ppm) for cinnamaldehyde for the 100 ppm object after caecal incubation was observed, which could indicate that this phytochemical was (totally) transformed under caecal conditions. The 1000 ppm object after incubation gave a moderate response but could not be quantified due to a lack of linear peak signal response.

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Essential fatty acids and tocopherols of evening primrose oil (*Oenothera biennis* L.)

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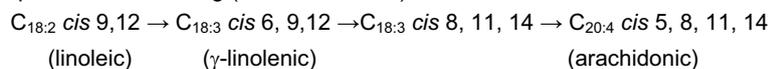
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Introduction

The possibility of production and use of γ -linolenic acid (GLA, C_{18:3} *cis* 6, 9, 12) is the object of extensive investigations lately. Namely, GLA is now recognized as an interesting material with beneficial health properties. Claims have been made for its use in the treatment of multiple sclerosis, arthritis, eczema, premenstrual syndrome and other ailments. It has been shown that dietetic supplementation with evening primrose oil results in formation of a whole spectra of metabolites affecting positively the function of a number of organs, cardiovascular system and different inflammations (Carter, 1988; Sarker, 2001; Kremer, 1996).

GLA is a biological intermediate in the conversion of freely-available linoleic acid to the important, but less readily available arachidonic acid. This change is a three-step process involving Δ^6 -desaturation, elongation and Δ^5 -desaturation, of which, the first step is rate-determining (Gunstone, 2004).



GLA is essential for persons with disturbed metabolism, where the transformation of linoleic to γ -linolenic acid is impossible. This depression of bio-conversion is especially expressed under stress. In such cases, the direct intake of GLA is recommended (Karleskind, 1996).

GLA is present in a number of seed oils, of which only three are commercially available: *Oenothera biennis* L. – evening primrose (typical result of GLA content is 10% wt), borage (23% wt) and blackcurrant (17% wt) (Gunstone, 2003, 2004).

The assortment of special oils on the European market, and broader, includes also evening primrose oil.

Evening primrose seed oil is separated by solvent extraction, or pressing. Due to interest of nutritionists and public, the demands of modern consumers are pointed to nonprocessed oils. For that reason natural oils, like virgin, e.g., cold pressed oils, where no processing (refining) was applied, are gaining popularity.

The nutritive value and oxidative stability of virgin evening primrose seed oil, obtained by cold pressing on screw press was investigated and presented in the paper.

Material and methods

Two samples of cleaned evening primrose seed (*Oenothera biennis* L.) were pressed on the screw press »Komet«, under conditions which enabled the temperature of obtained oil to be below 50°C. The oil content of samples was 23.45% (sample 1) and 26.03% (sample 2). The moisture content of seed was about 9%. The pressed oil was kept for three days at room temperature for sedimentation, than decanted and filtrated.

The following methods were used for the investigations: fatty acid composition – ISO 5508: 1990, content and composition of tocopherols – ISO 9936: 1997, iodine value – ISO 3961: 1999, peroxide value – ISO 3960: 1998. Oxidative stability of the oil was determined at 100°C using the Rancimat 617 apparatus (air flow 18–20 l/h) (ISO 6886: 1996) and at 63°C by Schaal oven test (Pokorny et al., 1985).

Results and discussion

Nutritive value of evening primrose oil

The fatty acid composition is among the most important characteristics of evening primrose seed oil, whereas the GLA content should be underlined, Table 1.

Table 1. Fatty acid composition of evening primrose seed oil

Fatty acid (% wt)	Sample	
	1	2
C _{16:0} palmitic	6.30	6.47
C _{18:0} stearic	1.59	1.35
C _{18:1} oleic	12.55	15.84
C _{18:2} ω-6 linoleic	70.83	73.46
<u>γ-C_{18:3} ω-6 (GLA)</u>	8.83	2.85
Saturated fatty acids (%)	7.89	7.82
PUFA* (%)	77.66	76.31
Total unsaturated fatty acids (%)	90.21	92.15
Ratio of unsaturated/saturated fatty acids	11.43	11.78
Iodine value (g/100 g)	147	148

* polyunsaturated fatty acids

The fatty acid composition of oils obtained from two samples of evening primrose seed is rather different. The content of GLA in sample 2 is very low, only 2.85%, while in sample 1 this content is significantly higher – 8.83%. According to results obtained by Hudson (Hudson, 1984), the content of GLA differs significantly in different *Oenothera* spp. The content of GLA was ranging from 2-20% in a series of 192 investigated samples. However, in 80% of samples, the content was 10 -11.9%. A characteristics of evening primrose seed oil is that the whole amount of linolenic acid is in γ form. This is the main difference from borage seed and blackcurrant oils, where the α -form is also present.

The content and composition of tocopherols of cold pressed evening primrose oil (sample 1) and some literature data are presented in Table 2.

Table 2. Tocopherols in evening primrose oil

Tocopherols	Cold pressed oil		Literature	
			Hudson, 1984	Eskin, 2002
	(mg/kg)	(%)	(mg/kg)	(mg/kg)
Total	282	-	263	454-664
α -tocopherol	149.9	53.16	76	99-356
β -tocopherol	3.05	1.08	-	
γ -tocopherol	123.7	43.86	187	298-358
δ -tocopherol	5.33	1.89	-	10-19

Cold pressed evening primrose seed oil contains 282 mg/kg of total tocopherols, whereas α - (53.16%) and γ - (43.86%) tocopherols are the most represented ones.

Oxidative stability of evening primrose oil

Evening primrose oil has a beneficial effect on human health, however, due to high content of polyunsaturated fatty acids (over 75%, Table 1), it is prone to oxidative deterioration, so the problem of oil stability is rather expressed, Table 3.

Table 3. Oxidative stability of evening primrose oil

	Induction period	PV* (mmol/kg)
Schaal oven test (at 63 °C)	5 days	22.67
Rancimat test (at 100 °C)	8.5 hours	-

*after 96 hours at 63 °C

Regarding the oxidative stability, evening primrose seed oil is very similar to cold pressed linoleic type sunflower oil. The peroxide value after four days of Schaal-oven test was 22.67 mmol/kg, while in sunflower oil this value is ranging from 22 to 28 mmol/kg (Kuc et al, 2004).

The induction period, obtained by Rancimat test, was 8.5 and 7.73 – 8.28 hours, for evening primrose oil and sunflower oil, respectively (Dimic, 2000).

Conclusion

Having in mind the high variability of GLA content, a special attention has to be paid when choosing evening primrose seed for the production of cold pressed oil. Due to the extremely high content of polyunsaturated fatty acids, evening primrose seed oil is prone to oxidative deterioration, so it has to be protected from oxidation.

Summary

The possibility of production and use of γ -linoleic acid (GLA) is the object of extensive investigations lately. Besides vitamins, minerals and other functional components, certain fatty acids have an important role in everyday diet also. GLA is essential for persons with disturbed metabolism, where the transformation of α -linoleic to γ -linolenic acid is impossible. This depression of bio-conversion is especially expressed under stress. In such cases, the direct intake of GLA is recommended. The paper points to the nutritive significance of evening primrose oil. The parameters which characterize the quality of oil, the fatty acid composition and iodine value were determined. The dominant fatty acids in cold pressed evening primrose oil are: palmitic, stearic, oleic, linoleic and γ -linolenic 8.83% - sample 1 and 2.85% - sample 2. The tocopherols were determined by HPLC method, and their total content was 282 mg/kg. The oxidative stability of oil is relatively poor, the induction period, determined by Rancimat test at 100 °C, was 8.5 hours.

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Bioactive compounds in tomatoes and tomato products – investigations of different commercially available foodstuffs

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Introduction

Tomatoes and tomato products are very popular in Germany and contribute for 22% to the total vegetable consumption. In 2003, consumption of tomatoes and tomato products per head and year was 18.6 kg (raw tomatoes 8 kg). In addition to carotenoids, tomatoes deliver considerable amounts of vitamin C and tocopherols. Recent epidemiological observations suggested that tomato consumption may have organspecific chemopreventive effects on some types of cancer and cardiovascular diseases (Agarwal and Rao 2000, Willcox et al. 2003). The non-provitamin A active carotene lycopene is one of the major phytochemicals in tomatoes contributing to the anti-carcinogenic function. Tomatoes are consumed mainly as processed tomatoes. Homogenisation as well as heat treatment of tomatoes disrupt cell membranes, with heat treatment being suggested to disrupt also the protein-carotenoid-complex (van het Hof et al. 2000). Therefore, processing increases the bioavailability of carotenoids in humans. In contrast, processing of tomatoes also leads to significantly decreased vitamin C concentrations in the products.

Materials and Methods

Aim of the research was to determine contents of carotenoids and vitamin C in 8 or 9 brands of raw tomatoes, tomato juices, tomato ketchups, tomato sauces, sieved tomatoes (tetra-pack) and pizza tomatoes (tin), reflecting the product diversity in the German market.

Samples were extracted with methanol/tetrahydrofuran (1+1, v/v) and analysed on carotenoids using C₃₀-HPLC with photodiode array detection modified to Böhm (2001). Vitamin C was measured spectro-photometrically (Speitling et al. 1992) after extraction with *meta*-phosphoric acid. The contents were compared using one-way analysis of variance (ANOVA). To determine differences between the mean values of the product groups the Tukey test ($p < 0.05$) was applied.

Results and Discussion

(*all-E*)-Lycopene, different (*Z*)-isomers of lycopene and (*all-E*)- β -carotene were detected in all samples of tomatoes and tomato products. Raw tomatoes additionally contained traces of (*all-E*)-lutein. Takeoka et al. (2001) observed that during processing lutein increased most, followed by lycopene and β -carotene.

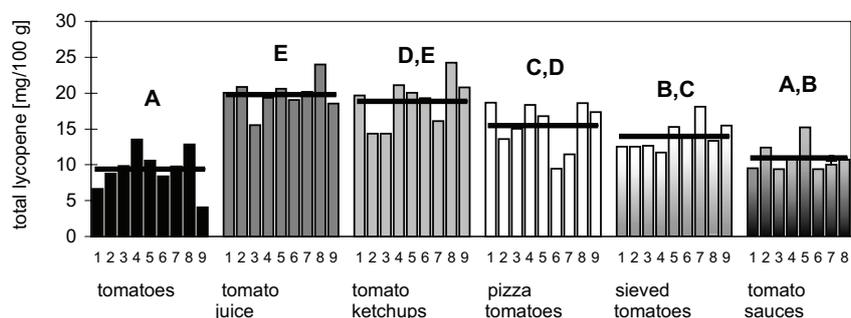


Figure 1: Contents of total lycopene in samples of tomatoes and tomato products and mean values of the product groups, mean values of the product groups showing the same index are not significantly different ($p > 0.05$)

Total lycopene concentrations in tomatoes ranged between 4.1 and 13.6 mg/100 g fresh matter, in processed tomato products between 9.4 and 24.8 mg/100 g fresh matter (**Figure 1**). The results indicate that raw tomatoes have lower mean lycopene concentrations compared to tomato products due to dehydration during the processing. This observation could also be explained by the use of lycopene-rich tomato varieties as raw material for tomato products (Abushita et al. 2000). The contents of lycopene in tomatoes can be affected by many factors such as variety, growth conditions and time of harvest.

Table 1: Mean relative contents of lycopene isomers [%] in samples of tomatoes and tomato products, values showing the same index are not significantly different ($p > 0.05$)

	tomatoes	tomato juices	tomato ketchups	pizza tomatoes	sieved tomatoes	tomato sauces
(<i>all-E</i>)-lycopene	95.1±1.5 ^a	94.0±1.0 ^a	93.6±2.0 ^a	94.6±1.9 ^a	93.1±0.7 ^a	81.7±10.1 ^b
Σ (<i>Z</i>)-lycopene	4.8±1.5	6.0±1.0	6.4±2.0	5.4±1.9	6.9±0.7	18.3±10.1

(*all-E*)-Lycopene is the predominant isomer in each sample (**Table 1**). (*all-E*)-Lycopene may undergo isomerisation during food processing, which in turn can affect the biological activity (Shi and Le Maguer 2000). The mean isomer ratios are not significantly different in tomatoes, tomato juices, tomato ketchups, pizza tomatoes and sieved tomatoes. Tomato sauces had significant more (*Z*)-isomers of lycopene caused by heating for any length of time in the presence of oil (Agarwal et al. 2001).

β -Carotene in tomatoes and tomato products was analysed in amounts of 0.1 - 1.8 mg/100 g fresh matter (**Figure 2**). Contrary to lycopene, β -carotene was significantly higher in raw tomatoes compared to processed tomatoes. The results indicate that β -carotene is less resistant against food processing including homogenisation and heat treatment than lycopene.

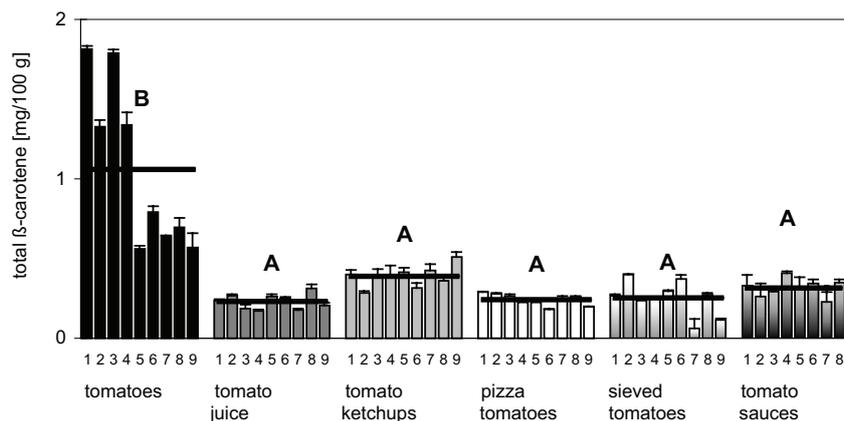


Figure 2: Contents of total β -carotene in samples of tomatoes and tomato products and mean values of the product groups, mean values of the product groups showing the same index are not significantly different ($p > 0.05$)

Vitamin C ranged between 0.3 and 42.3 mg/100 g fresh matter (**Figure 3**). Compared to untreated tomatoes, processed products contained lower contents of vitamin C due to its lability. Pizza tomatoes showed the lowest vitamin C values (0.3 - 5.9 mg/100 g). The contents are not great when compared to cabbages, leafy vegetables or citrus fruits, but considering the quantities of consumed tomatoes and tomato products this species plays an important role in the total consumption of vitamin C.

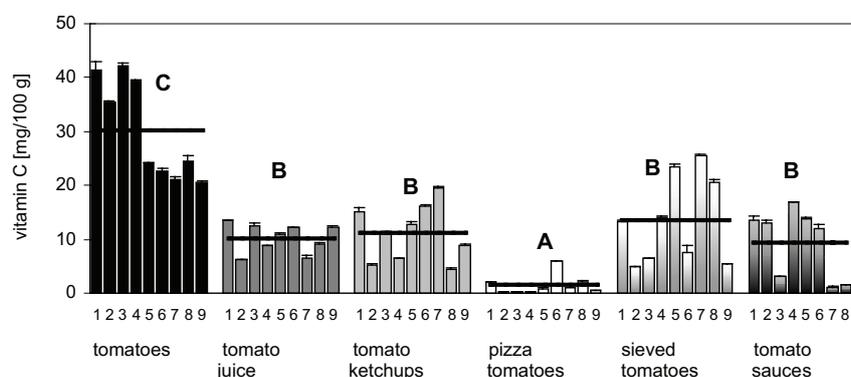


Figure 3: Contents of vitamin C in samples of tomatoes and tomato products and mean values of the product groups, mean values of the product groups showing the same index are not significantly different ($p > 0.05$)

For the dietary relevance of the results it must be pointed out that in the majority of cases consumers heat pizza tomatoes, sieved tomatoes and tomato sauces before ingestion. In contrast, tomatoes, tomato juice and tomato ketchup are usually consumed without other processing. Further processing of tomato products can result in a change of the tomato ingredients. In terms of the increased bioavailability of carotenoids contrary to the reduced vitamin C levels of processed tomatoes, a combination of raw tomatoes and processed tomato products is recommended for a balanced diet.

Acknowledgements

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Vitamin C and antioxidative potential of oranges and other citrus fruits

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Introduction

Dietary antioxidants present in fruits and vegetables contribute to the prevention of degenerative diseases like cardiovascular diseases and cancer by counteracting oxidative damage. Thus, the aim of this investigation was to determine the contents of vitamin C (ascorbic acid) and total phenolics in combination with the antioxidant capacity of different citrus fruits as well as the respective contribution of vitamin C to the sum parameters. In addition, the effect of frozen storage at $-18\text{ }^{\circ}\text{C}$ on the content of vitamin C was ascertained.

Materials and Methods

Edible parts of 12 samples of citrus fruits from different supermarkets, with nine oranges (same cultivar, except for orange 3), one lemon, one lime and one grapefruit, were homogenised and analysed after a frozen storage period of 14 months. Vitamin C was also measured prior to that. Vitamin C was determined photometrically using 2,4-dinitrophenylhydrazine (Speitling et al. 1992, Vazquez Oderiz et al. 1994). The content of total phenolics was assessed photometrically by using the Folin-Ciocalteu method (Schlesier et al. 2002). Antioxidant capacity was investigated by using the ferric reducing antioxidant power (FRAP) assay and the Trolox equivalent antioxidant capacity (TEAC) assay (Schlesier et al. 2002). Furthermore, the contribution of vitamin C to the sum parameters total phenolics content und antioxidant capacity was determined.

Results and Discussion

Oranges had the highest content of vitamin C (46-56 mg/100 g) compared to the other investigated citrus fruits, one orange sample even contained 72 mg/100 g. The lowest Vitamin C content was determined in lime (37 mg/100 g) (**Figure 1**). After 14 months of frozen storage only two orange samples showed a significant decrease in

vitamin C content. All other citrus samples lost less than 10% of their content of original vitamin C (data not shown).

Total phenolics contents expressed as gallic acid equivalents (GAE) were similar in all fruits tested (61–81 mg GAE/100 g) with grapefruit showing the highest content (**Figure 1**). Phenolic constituents of citrus fruits are cinnamic acids like caffeic acid, ferulic acid, *p*-coumaric acid and sinapic acid as well as flavanoid glycosides such as hesperidin and naringin with the last one occurring mainly in grapefruit (Kanitsar et al. 2001). Especially in grapefruit there was a high contribution of phenolic compounds other than vitamin C to the total phenolics content (70-77%). Altogether vitamin C contributes about one third to the total phenolics content in the citrus fruits investigated (**Figure 2**).

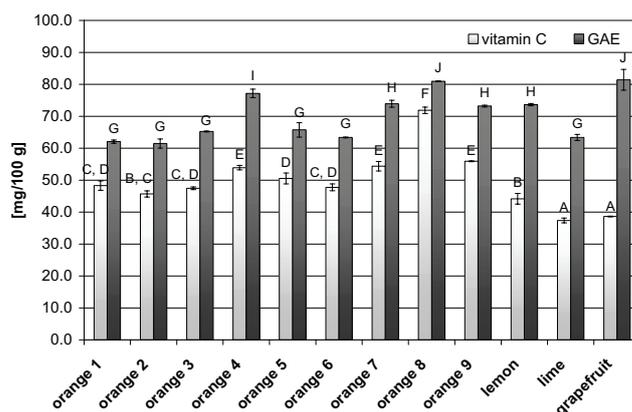


Figure 1: Contents of vitamin C and total phenolics (expressed as GAE) of all assayed fruit samples. Bars with same letters are not significantly different ($p > 0.05$).

The highest antioxidant capacity was determined in lemon (FRAP: 0.71 mmol/100 g, TEAC: 0.35 mmol/100 g) compared to the average of all orange samples investigated, the lowest one in lime (FRAP: 0.46 mmol/100 g, TEAC: 0.21 mmol/100 g). Orange sample 8 was an exception with a high FRAP value (0.76 mmol/100 g) and TEAC value (0.37 mmol/100 g) (**Figure 3**). In oranges at least 78% of the antioxidant capacity is caused by vitamin C (data not shown), but factors that perhaps affect the measurement of the antioxidant capacity are not completely investigated.

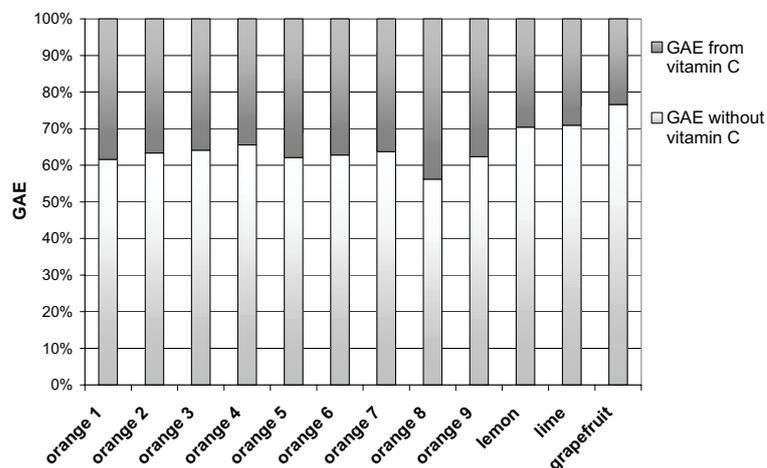


Figure 2: Contribution of vitamin C and other phenolic compounds to the total phenolics content expressed as GAE.

There was a significant correlation between the results of the two antioxidant capacity assays ($r = 0.887$, $p < 0.01$) whereas only low relationships were found between the contents of vitamin C, total phenolics and antioxidant capacity.

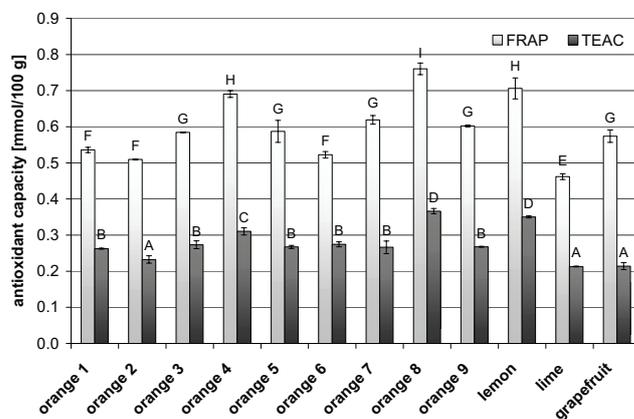


Figure 3: Antioxidant capacity expressed as FRAP and TEAC values. Bars with same letters are not significantly different ($p > 0.05$).

Conclusions

Because of their high contents of vitamin C and other antioxidative compounds citrus fruits enrich human nutrition, especially with regard to the prevention of diseases resulting from oxidative damage. However, remarkable differences between fruits from several supermarkets can emerge, depending on cultivation, degree of ripeness and environmental conditions of the particular batch. The content of vitamin C in homogenised fruits alters little during frozen storage over many months. To further clarify the differences between several citrus fruits, studies with a larger number of fruit samples are required.

Summary

The intention of this study was to ascertain the antioxidant capacity of different citrus fruits after frozen storage including the quantitative analysis of vitamin C and total phenolics. From the investigated fruits oranges had the highest content of vitamin C while lemon showed the highest antioxidant capacity, except one single orange sample. Total phenolics content was similar in all fruits tested, but the contribution of vitamin C to this parameter varied. Frozen storage over several months had little effect on the vitamin C content. Remarkable differences between fruits from several supermarkets point out the influence of cultivation, degree of ripeness and environmental conditions on various ingredients.

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Urinary excretion of antioxidants following consumption of *Hibiscus sabdariffa* L. extract

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Introduction

Hibiscus sabdariffa L. is a traditional Chinese rose tea and is used effectively in native medicines against hypertension, inflammation, and liver disorders (Chen et al., 2003). The calyces of *Hibiscus* species contain several antioxidant compounds like phenolic acids, flavonoids, and especially anthocyanins (cyanidin- and delphinidin-glycosides) (Chen et al., 2003; Tsai et al., 2002). The aim of the present pilot study was to assess the renal excretion of *Hibiscus sabdariffa* L. extract (HSE) antioxidants in one healthy volunteer.

Materials and Methods

After an overnight fast, one healthy non-smoking volunteer (female, 24 years, BMI 25.3 kg/m²) received a bolus quantity of 200 ml of HSE (containing 217 mg anthocyanins and 263 mg total phenolics, equivalent to an antioxidant capacity of 3.20 mmol [FRAP assay], 2.03 mmol [PCL assay], and 3.30 mmol [TEAC assay]), or water as an antioxidant-free control beverage, together with a standardised breakfast. Urine samples were collected predose and in 6 intervals up to 24 h after dosing (0-2, 2-4, 4-6, 6-8, 8-10, and 10-24 h). During the experimental period, only the consumption of water and of two further standardised meals (lunch and dinner) was allowed.

Analytical Procedure: Anthocyanins and metabolites (HPLC-DAD/ LC-ESI-MS/MS; Netzel et al., 2001; Kammerer et al., 2004), total phenolics (photometrical; Singleton & Rossi, 1965), PCL (Popov & Lewin, 1999), FRAP (Benzie & Strain, 1996), TEAC

(Re et al., 1999), hippuric acid (HPLC-UV/ LC-ESI-MS/MS; Kubota et al., 1988; Kammerer et al., 2004), ascorbic acid and uric acid (HPLC-UV; Ross, 1994; Vazquez-Oderiz et al., 1994), were determined in the urinary samples of the volunteer.

Results

After HSE ingestion, delphinidin-3-sambubioside, cyanidin-3-sambubioside, and delphinidin-monoglucuronide (metabolite) were excreted in the volunteer' urine (0.031 % of the administered dose) and were unambiguously identified by HPLC-ESI-MS/MS. Compared to the ingestion of water, HSE consumption resulted in an increased urinary excretion of total phenolics (+12 %), other antioxidant compounds (+13 % [FRAP assay], +22 % [PCL assay], +36 % [TEAC assay]), and hippuric acid (potential colon metabolite; +104 %) within 24 h (Table 1 and Figures 1-3).

Table 1. Summary of urinary excretion data of antioxidants and metabolites following ingestion of a single oral dose of 200 ml HSE or 200 ml water to one healthy adult, respectively.

parameters/ substances	dose	urine	
		Control	Hibiscus
FRAP	3.20 mmol/subject	1.34 mmol/24 h	1.51 mmol/24 h
PCL	2.03 mmol/ subject	0.89 mmol/24 h	1.09 mmol/24 h
TEAC	3.30 mmol/ subject	3.28 mmol/24 h	4.47 mmol/24 h
total phenolics	262.9 mg GAE/ subject	253.8 mg/24 h	284.4 mg/24 h
anthocyanins and metabolites	217.4 ¹ mg/subject	nd	62.4 ¹ µg/24 h 4.9 ² µg/24 h
hippuric acid	na	57.7 mg/24 h	117.6 mg/24 h

¹sum of delphinidin-3-sambubioside and cyanidin-3-sambubioside; ²delphinidin-monoglucuronide; GAE = Gallic Acid Equivalents; na = not analysed; nd = not detectable; total phenolics, FRAP-, PCL- and TEAC-values are corrected for ascorbic acid and uric acid.

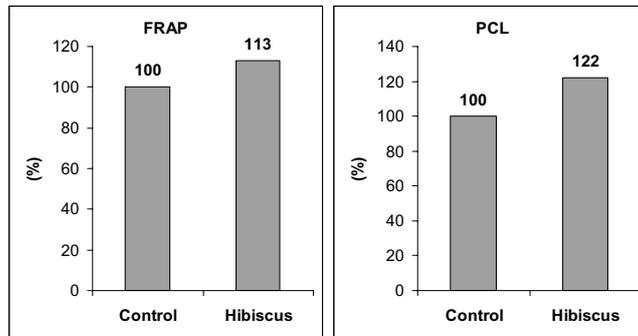


Figure 1. Changes (Hibiscus vs. Control) in urinary excretion of antioxidants measured as FRAP and PCL, respectively (control values are calculated as 100%).

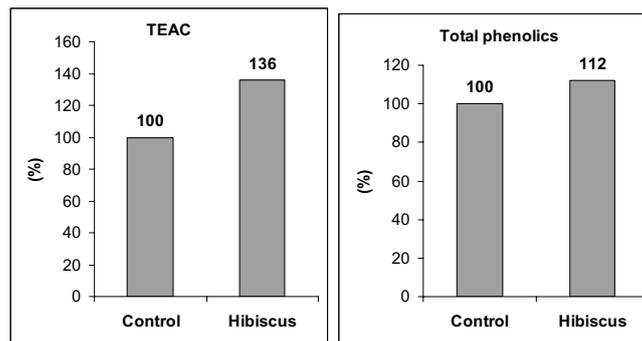


Figure 2. Changes (Hibiscus vs. Control) in urinary excretion of antioxidants measured as TEAC and total phenolics, respectively (control values are calculated as 100%).

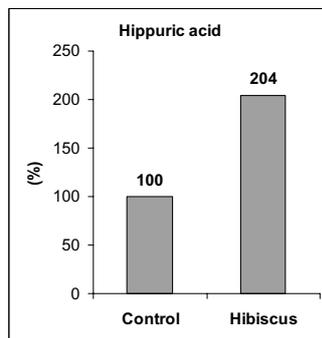


Figure 3. Changes (Hibiscus vs. Control) in urinary excretion of hippuric acid (control values are calculated as 100%).

In **conclusion**, the results of this pilot study may indicate an *in vivo* antioxidant effect of *Hibiscus* antioxidants in healthy humans.

Summary

The aim of the present pilot study was to assess the renal excretion of *Hibiscus sabdariffa* L. extract (HSE) antioxidants in one healthy volunteer. After a single oral dose of 200 ml of HSE, the parameters obtained in urine were compared to those obtained after the ingestion of water (control). Urine was collected in 6 intervals up to 24 h post-intake. After HSE ingestion, unchanged anthocyanins and one monoglucuronide were excreted in the volunteer's urine. Compared to the ingestion of water, HSE consumption resulted in an increased urinary excretion of total phenolics, other antioxidant compounds, and hippuric acid (potential colon metabolite) within 24 h. In conclusion, the results of this pilot study may indicate an *in vivo* antioxidant effect of *Hibiscus* antioxidants in healthy humans.

Acknowledgement

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Oxidative stability of virgin olive and high oleic sunflower oil

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Introduction

The statement that certain lipids have specific physiological effect on human organism, affected the interest of scientists and public for their use, for the maintaining or improvement of health. For that reason, the demands of modern consumer are pointed to natural, cold pressed oils. Due to the fact that cold pressed oils are not refined, their typical flavour, and natural nutritive value remain unchanged (Dimic et al., 2003). Olive oil, with eldest tradition, is the typical representative of cold pressed oils. However, lately, the interest to broaden the assortment of natural edible non-refined oils is increasing towards oils obtained from other raw materials. The virgin cold pressed sunflower oil is of special interest.

The standard sunflower oil is very »healthy« due to high content of linoleic acid (about 65%) and vitamin E (~ 700 mg/kg) (Kuc et al., 2003), new hybrids were developed worldwide, with very high content of oleic acid (Gunston, 2003).

Due to the demands from the market and nutritionists, our selectioners also developed an oleic type sunflower hybrid, NS Olivko. The efforts for the introduction of this hybrid in mass production and bigger production of cold pressed oil are intensive in our country.

Since the fatty acid profile of high-oleic sunflower oil and olive oil are very similar, the aim of the work was to compare the quality, nutritive value and stability of these two kinds of oil, as typical representatives of their category.

Material and methods

The cold pressed sunflower oil, obtained under laboratory conditions, was used for the investigation. Cleaned whole seed, moisture content 8,5%, was pressed on the laboratory screw press »Komet«. The temperature of pressed oil, at the outlet of the press, was about 40°C. The pressed oil was kept the 3 days at room temperature (20-25°C) to sediment, after that decanted and filtered.

Olive oil, two samples, was taken from the market, by random choice. The samples were produced in Italy and Spain.

Parameters of quality and nutritive value were determined by ISO methods. Stability was determined by Schaal oven test at 63±2°C (Pokorny et al., 1985) and Rancimat test at 120°C (air flow 18-20 l/h) (ISO 6886:1996). Pigments were determined according to Wolff (Wolff, 1968).

Results and discussion

Nutritive value and quality of oil

The content of monounsaturated oleic fatty acid (Table 1) is very high in all investigated samples, about 80%, while the total content of unsaturated fatty acids is the highest in sunflower oil – 93,35%. The content of saturated fatty acids of olive oil samples is 2,6 – 2.8 times higher compared to sunflower oil.

Table 1. Nutritive value and quality of cold pressed high-oleic sunflower and virgine olive oil

Characteristic	Cold pressed sunflower oil	Extra quality virgin olive oil	
		Italy	Spain
FA* composition (%)			
C 16:0	4.80	11.09	14.26
C 18:0	1.52	0.99	1.12
C 18:1	79.09	80.28	80.55
C 18:2	14.26	5.189	2.00
C 20:0	traces	0.29	0.31
Total saturated FA	6.32	13.72	16.30
Total unsaturated FA	93.35	86.28	83.54
Total tocopherols (mg/kg)	799	266	88
Chlorophylles (mg/kg)	7.62	23.75	26.96
FFA** (% oleic acid)	0.37	0.42	0.81
PV*** (mmol/kg)	1.99	7.26	2.88

* fatty acids ** free fatty acids *** peroxide value

Significant differences were found in total tocopherols content depending on oil kind. Cold pressed sunflower oil contained more tocopherols (2.5 to 9 times) than the olive oil samples. As the portion of α -tocopherols in both kinds of oils is about 95% (Kuc et al., 2003), it is obvious that the content of vitamin E is significantly higher in cold pressed sunflower oil. Having in mind these facts, from the standpoint of fatty acid composition and vitamin E content, the nutritive value of high-oleic sunflower oil is higher.

The colour of edible nonrefined oils is expressed, depending on the amount of pigments. Olive oil contains significantly more pigments, chlorophylls and carotenoids, hence it is usually rather dark.

Regarding acidity, olive oil samples are in the category of extra quality. However, the free fatty acids content of sunflower oil is lower. At the same time, the peroxide value was also the lowest in this oil. On the basis of these two chemical parameters, the quality of cold pressed high-oleic sunflower oil is better, compared to extra virgin olive oil.

Oxidative state and stability of oils

Besides quality, the stability of edible oils is also very important, both for use and from the health-nutritive aspect.

Table 2. Oxidative state and shelf life of high-oleic sunflower and olive oil

Characteristic	Cold pressed sunflower oil	Extra quality virgin olive oil	
		Italy	Spain
$A_{232}^{1\%}$	1.94	2.13	1.83
$A_{270}^{1\%}$	0.16	0.14	0.20
R value (A_{232}/A_{270})	12.08	15.21	9.15
Rancimat test Induction period (h)	4.15	7.00	8.06

Considering the content of primary oxidative products (Table 1) and specific absorbances (Table 2), the oxidative changes are less expressed in sunflower oil, however, the stability of olive oil is better. The induction period of olive oil samples, determined at 120°C, is 7 and 8.06^h, and of sunflower oil 4.15 hrs. The difference is more pronounced at lower temperatures (Figure 1). Namely, the induction period at 63°C, is 22 days for sunflower oil, 28 days for olive oil from Spain and more than 45 days for oil from Italy.

Different polyphenolic compounds, naturally present in olive oil, with strong antioxidative effect, contribute to stability of this oil. According to data presented in the literature, the content of phenolic substances in olive oil ranges from 100 to 150 mg/kg. The strongest antioxidative action is associated with hydroxytyrosol (Haumann, 1996). However, during the production of cold pressed sunflower oil, the majority of phenolic compounds remains in the cake (De Leonadis et al., 2003).

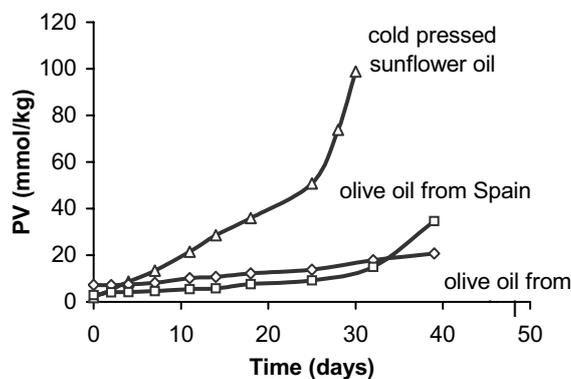


Figure 1. Oxidation curves of cold pressed high oleic sunflower and extra virgin olive oil

Conclusion

The cold pressed oil obtained from NS-Olivko sunflower, with high oleic-acid content is very similar to, or even better than the extra virgin olive oil. However, although the fatty acid composition of the two kinds of oils is similar, the stability of olive oil is better.

Summary

The composition, quality, nutritive value and stability of virgin oil obtained of high oleic type sunflower hybrid NS-Olivko were determined and compared to extra quality virgin olive oil. The cold pressed sunflower oil, containing 80% of oleic acid is very similar, or even better than the olive oil concerning several quality characteristics (lower peroxide value, lower acidity, higher vitamin E content). However, despite of very similar fatty acid composition, virgin olive oil has much better oxidative stability.

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Influence of vitamin E and vitamin C on the antioxidative status in muscle and meat quality of pigs

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Introduction

Vitamin E and C are primary antioxidants in biological systems and break the chain of lipid peroxidation. Many studies suggest that vitamin C and vitamin E act synergistically (Gey, 1998). The pig synthesizes vitamin C and does not require it in the diet, but there remains the possibility that endogenous synthesis is inadequate to maximize vitamin C's contribution to oxidative stability. Datas from pig experiment suggest vitamin C supplementation before slaughter (Kremer et al., 1999) and injected in muscle postmortem (Gimeno et al., 2001) can improve parameters of meat quality. The purpose of this study was to evaluate further the effects of vitamins E and C supplementation before slaughter and by injection in muscle postmortem on level of α -tocopherol and L-ascorbic acid and lipid peroxidation status in fresh and chill-stored pork and on some meat qaulity parameters.

Material

Animals: Thirty Slovak White Meaty pigs (DNA tested on malignant hyperthermia)

Feeding: Control group was fed a diet supplemented with basal level α -tocopherol. Experimental groups received a supplemental level vitamin E (ROVIMIX[®] E-50 SD), (500 mg α -tocopheryl acetate /kg) for 30 days before slaughter (group E, n = 10) and a supplemental level vitamin E (500 mg α -tocopheryl acetate /kg) and vitamin C (ROVIMIX[®] STAY-C[®] 35), (200 mg ascorbic acid /kg), (group EC, n = 10) for 30 days before slaughter. (Table 1).

Sampling: After slaughter (110 kg), the carcasses were chilled at 4°C for 24 h, then longissimus dorsi (part lumborum, LD) muscle was removed from each carcass. A portion of the sample was used immediately (24 h) and wrapped in aluminium film and stored in a refrigerator at 4°C for 5 days until analysed. A portion of LD from control and with vitamin E supplemented pigs was injected with 10% by weight of solution 1.5% Ca Ascorbate (Sigma-Aldrich) and wrapped in aluminium film and stored in a refrigerator at 4°C for 5 days until analysed.

Methods

Chemical analysis: The concentration of vitamin E (α -tocopherol) was measured by HPLC. For vitamin C (ascorbic acid) the methodology with 2,4-dinitrophenylhydrazin as a color reagent was used. Results were expressed as mg/kg dry matter (mg/kg DM). For evaluating the stability of the skeletal muscle (fresh samples) lipids against stimulated lipid peroxidation by Fe^{2+} /ascorbate a determination of thiobarbituric acid reactive substances (TBARS) was performed. Thiobarbituric acid reactive substances (TBARS) were expressed in terms of malondyaldehyde (MDA, mg/kg tissue, or nmol/mg protein).

Meat quality measurements: The pH value of the carcass (45 min post mortem), drip loss, colour changes (Miniscan, L, a) after refrigerated storage (24 h, or 5 day) were measured. Shear force was determined in cooked samples (internal temperature 75°C) with Warner-Bratzler (W-B) apparatus.

Results and discussion

The levels of α -tocopherol (Fig. 1) are higher or comparable with previously reported results (Buckley et al., 1995). We received a significantly lower drip loss value ($P < 0.05$) in pigs supplemented with vitamin E and vitamin C (Tab 1). Also Kremer et al. (1999) introduced vitamin C supplementation before slaughter of pigs can improve drip loss value of meat. A beneficial effect of dietary vitamin E on the oxidative stability of pork chops (Fig. 3, 4) and ground meat after storage was also shown by others (Nuernberg et al., 2002).

A higher „a“ value in Ca ascorbate injected muscle after a five days chill storage (Table 2) could be connected to a reducing activity of ascorbic acid on metmyoglobin as was discussed also by Lee et al. (1999). The illustrated differences (Fig. 5) substantiate the protective action of feed supplemented with vitamin E and/or vitamin C incorporated into the muscle tissue against peroxidation. It has been stated that additional vitamin C can either exhibit antioxidative or prooxidative effects (Wong et al., 2005).

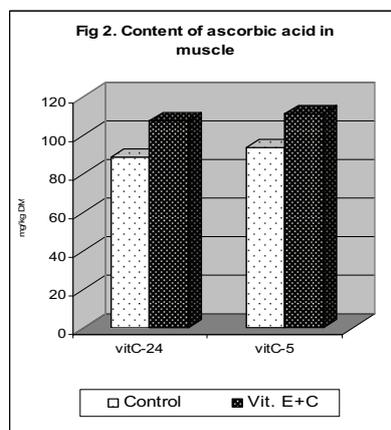
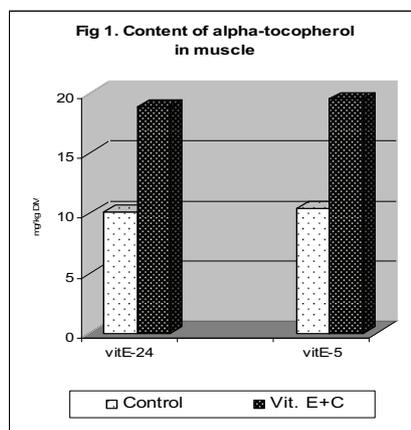


Table 1. Pork quality (*m. longissimus dorsi*)

Trait	Time	Control		Vitamin E		Vitamin E+C	
		mean	S.D.	mean	S.D.	mean	S.D.
pH	45 min	6.27	0.22	6.38	0.19	6.45	0.26
Colour (L)	24 h	48.67	3.64	48.58	2.36	48.60	2.14
Drip loss, %	24 h	4.86 ^a	1.03	4.12	1.05	4.05 ^b	0.88
Shear force, kg	5 day	4.09	1.15	4.82	0.71	4.66	0.66

Means with different superscript letter differ (P<0.05)

Table 2. Effect of calcium ascorbate injection on meat quality (*m. longissimus dorsi*, 5 days storage)

Trait	Control	Cont. + Vit. C	Vit. E	Vit. E+C	SE
Ca, mg/kg	0.168 ^a	0.233 ^b	0.156 ^a	0.214 ^b	0.011
Vit. C, mg/kg	n.d.	274.8	n.d.	277.9	12.0
Colour L*	51.34	50.56	50.61	50.42	0.837
a	2.29 ^a	3.05 ^b	2.42 ^{a,b}	3.00 ^{a,b}	0.262
TBARS, mg/kg	0.290 ^a	0.287 ^a	0.235 ^b	0.259	0.015
W-B, kp	5.18	4.98	4.94	4.75	0.209

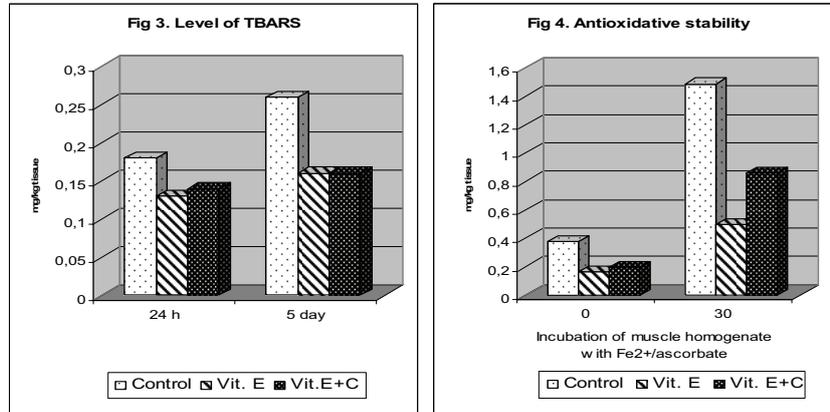
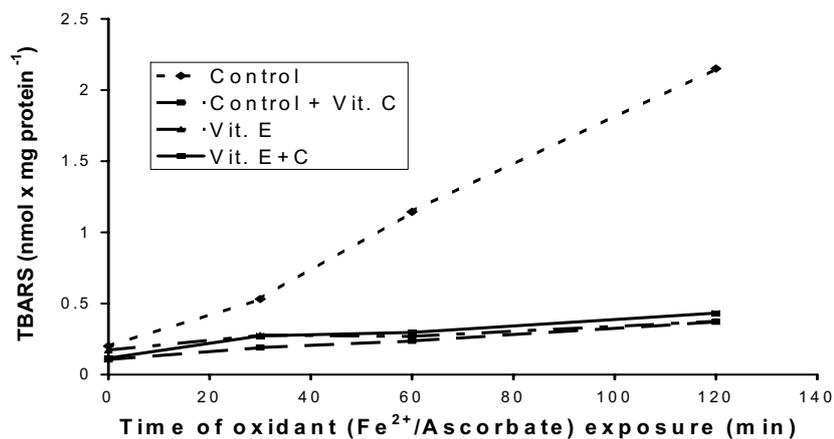


Fig 5. Peroxidative stability of longissimus muscle homogenate



Conclusions

Dietary supplementation (30 days) of vitamin E (500 mg α -tocopheryl acetate/kg feed) and vitamin C (200 mg/kg feed) to grow-finishing pigs:

- increases the concentrations of α -tocopherol and ascorbic acid in meat (longissimus dorsi).
- improve meat quality parameters (drip loss, pH).
- lipid oxidation measured as TBARS (MDA) and antioxidative capacity (Fe^{2+} /ascorbate induced) of meat can be positively influenced by supplementation of vitamin E.

Water soluble antioxidant calcium ascorbate (1.5 %), injected (10 % by weight) in meat:

- increases the concentration of calcium and ascorbic acid.
- stabilize the colour
- improve the antioxidative capacity.

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Utilization of flavonoids from black current seeds

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Introduction

Recently nutrition science is moving away from the classic concept avoiding nutrition deficiencies and to recommending a general appropriate nutrition and follows new concepts of “positive” and “optimal” nutrition. The reasons for the rethinking are the increasing scientific evidences supporting the thesis that several foodstuffs and food components possess an additional positive physiological and psychological effect, exceeding the only supply with basic nutrients. In this connection the concept of functional foods has been developed. Functional foods are defined as parts of usual nutrition which are able to improve well-being on the one hand and to lower the risk for certain diseases on the other hand. Today the market volume for functional foods is estimated worldwide to about 62 billions US \$. For 2005 sales increases to 75 billion US \$ are expected and according to HAAS (2000) the market potential on a long-term basis can even reach up to 230 billion US \$.

In the scope of the presented research project, which deals with the “utilization of flavonoids from black current seeds”, new functional foods with additional health benefit for the food sectors of baker’s products and sweets are developed. In this context the fruit seed press cakes from black currents are added to several products like bread, crispbread or fruit bars which are already established on the food market. Afterwards a sensory characterization of these food products is carried out. The fruit press cakes used are by-products of the fruit processing process. Beside poly unsaturated fatty acids, lipid soluble vitamins, phytosterols, proteins, minerals and fibres they contain high amounts of phenolic constituents. Because of their versatile spheres of activity and the consequential health advantage for the human organism, phenolic components have been the scope of numerous scientific investigations. The characterization of the health potential of the phenolic constituents located in the press cakes and therefore the confirmation of the health revaluation of the developed foods are carried out within the scope of the realization of an intervention study and the evaluation of many further scientific in-vitro and in-vivo investigations, what is nevertheless not the object of the here demonstrated executions. For the representa-

tive handling of the topic it is necessary to develop and evaluate analysis methods, which can be used for the characterization of the press cakes as well as the developed functional food concerning their content and compositions of phenolic components. In the following the developed methods as well as the first results of the investigations are shown and discussed.

Materials and Methods

The press cakes of the black current seeds have been provided by the Institute for Cereal Processing (IGV) Potsdam, Germany. The determination of the total polyphenol content was accomplished according to Singleton (1965). The extinction was acquired photometrically after conversion with Folin reagent at 750 nm and for the quantification catechin was used as reference substance.

To selectively extract the anthocyanes from the press cakes the procedure of the accelerated solvent extraction (ASE) has been optimized as follows:

Table 1: ASE- Method parameters for the extraction of anthocyanes

Preheating time:	0 min
Heating time:	5 min
Static extraction time:	20 min
Flush volume:	120 %
Purge time:	100 sec.
No. of cycles:	3
Extraction pressure:	15 MPa
Extraction temperature:	60°C
Extraction solvent:	water/ethanol (1:1 v/v)

The quantification of the anthocyanes was performed according to COSTA et al. (1998), those of the flavonoles, flavanoles and phenolcarboxylic acids according to Ichyanagi et al. (2000) by means of the capillary zones electrophoresis (CZE). After suitable optimization the following methods could be set up and validated (table 2/3).

Table 2: CZE - Method parameters for the determination of the anthocyanes

Instrument:	CE 1100 HP
Capillary:	fused-silica 64,5 cm (56) x 50 µm
Injection:	hydrodynamic, 50 mbar
Voltage:	25 kV
Injection time:	8 s
Buffer:	25 mM Na H ₂ HPO ₄ -25 mM Na ₂ HPO ₄ -30 % Acetonitril; pH 1,5
Temperature:	20 °C
Analysis time:	25 min

Table 3: CZE - Method parameters for the determination of flavonoids/ phenolcarboxylic acids

Instrument:	CE 1100 HP
Capillary:	fused-silica 80,5 cm (72) x 50 µm
Injection:	hydrodynamic, 50 mbar
Voltage:	25 kV
Injection time:	8 s
Buffer:	80 mM Borax – 7,5 mM CyDTA; pH 7,6
Temperature:	25 °C
Analysis time:	32 min

Results

As shown in the following table 4, the total polyphenol content of the press cakes is 0.37% on average. With regard to the already manufactured products the crispbread supplied with press cake and seeds of black currents were found to have the highest content of polyphenols with 0.22% (table 4).

Table 4: Total polyphenol contents in percent

black current seed – press cakes	crispbread			
	control	with seeds	with press cakes	with press cakes and seeds
0,37%	0,15 %	0,17 %	0,19 %	0,22 %

For the characterization of the press cakes and the manufactured food products two capillary electrophoresis methods have been developed and validated which reliably and reproducibly characterize the anthocyanes and flavonoids of the press cakes qualitatively as well as quantitatively. The reason for developing two separate analysis methods for the determination of anthocyanes on the one hand and flavonoids on the other hand is the fact, that flavonoids and phenolcarboxylic acids can be optimally separated under basic conditions (Frazier et al. 2003), while anthocyanes naturally exist under acidic conditions and are increasingly diminished under pH values higher than 7. At basic pH anthocyanes undergo a ring opening of the molecule, whereby through the intermediate stage of an α -dicetone an aldehyde and a phenoliccarboxylic acid are generated (Belitz et al. 2001).

Within the scope of the determination of the anthocyanes contained in the press cakes 4 main components were unambiguously identified on the basis of their retention times and their DAD-spectra compared to commercial reference substances (figure 1). Beside the components mentioned further minor components belonging to the group of the anthocyanes could be found, which however, could not be identified clearly. The amounts of anthocyanes ascertained for the press cakes (0.16 - 0.39%) correspond to the references for fresh black currents (\varnothing 0.25%).

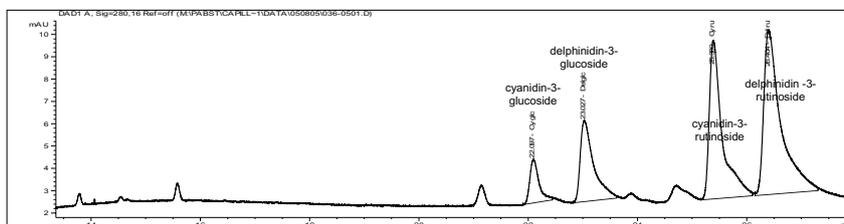


Figure 1: Electropherogram of the anthocyanes of black current seed press cakes

With the method optimized for the separation of the flavonoids the following reference substances can be separated reproducibly: kampferol-3-rutinoside, kampferol-3-glucoside, (-)-epigallocatechin, (+)-gallocatechin, (-)-epicatechin, (+)-catechin, quercetin-3-rutinoside, quercetin-3-glucoside, ferulic acid, chlorogenic acid, p-cumaric acid.

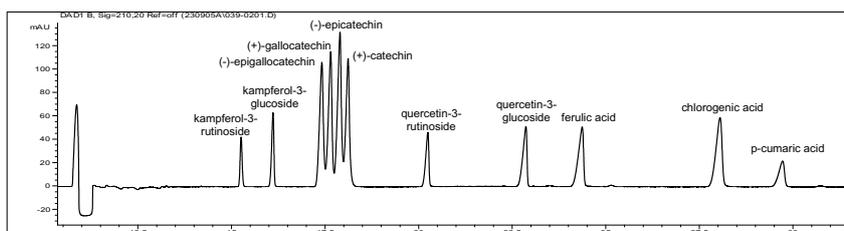


Figure 2: Electropherogram of the flavanol-, flavonol-, phenol carboxylic acids standards

Summary

For the characterization of the press cakes of black current seeds and the developed functional foods two capillary electrophoresis analysis methods have been developed and validated. With the help of these methods it is possible to determine the qualitative and quantitative content of anthocyanes on the one hand and flavonoids and phenolcarboxylic acids on the other hand.

The addition of black current seed press cakes to crispbread can contribute to the functional revaluation of the product, because, as shown by the results of the determination of the total polyphenolic content, the stability of the components is to the present level of knowledge unaffected by technological processing processes.

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Cell protective effects of wheat bran arabinoxylans – *in vitro* investigations with human colon cells

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Arabinoxylans are the most important dietary fibre components of wheat bran. It was the aim of this study to characterise chemoprotective effects of aqueous solutions of wheat bran fractions, namely water extractable (WeAx) and alkali extractable arabinoxylans (AeAx), and their products formed during the gut-flora-mediated fermentation in human colon cells. For this WeAx and AeAx were isolated from wheat bran, dissolved in cell culture medium or fermented with human faeces to produce fermentation supernatants (FS), which were analysed for content of short chain fatty acids (SCFA) using HPLC. Human colon HT29 cells were treated with the samples and we investigated possible antiproliferative activities by quantifying residual DNA with a fluorescent dye. Viability was determined by trypan blue exclusion and DNA damage using the comet assay, to assess possible antitoxic and antigenotoxic effects. The glutathione S-transferase (GST) activity was determined spectrophotometrically. Key results were that the fermentation of the arabinoxylans resulted in a significant increase of SCFA. Growth of the HT29 cells was efficiently inhibited by all FS including the control. A short term pre-incubation with WeAx decreased the level of H₂O₂ (75 µM) induced DNA damage significantly by 67 %. Fermented WeAx or AeAx were less protective. In contrast, all fermented samples reduced the genotoxicity of the lipid peroxidation product HNE (200 µM). We measured a significant induction in GST activity, which may point to an improved detoxification of HNE. Our results indicated that wheat bran ingredients before and after fermentation may act antiproliferativ and antigenotoxic, thus demonstrating chemoprotective properties in human colon cells.

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Carob fibre and Gallic Acid – Impact on proliferation parameters in human colon cell lines

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Introduction: Carob fibre is a food ingredient from the Mediterranean carob pod (*Ceratonia siliqua L.*). During a mild procedure soluble carob constituents are mainly removed by water extraction and the insoluble dietary fibre is retained in the residue, in addition to tannins and other polyphenols. Carob fibre was investigated for its ability to modulate a variety of different cellular parameters of proliferation using the human colon carcinoma cell line HT29 and the human colon adenoma cell line LT97.

Methods: For this, an aqueous extract of carob fibre was prepared (100 g carob fibre per 1 litre cell culture medium) and cells were incubated with 0.05 g to 20 g carob fibre per 1 litre cell culture medium. After 12, 24, 48 and 72 h treatment, growth and survival of the cells were measured by determining metabolic activity (reduction of resazurin) and cell number (intercalation of fluorochrome DAPI into DNA of remaining cells). Results were calculated in percentage of the medium control and the metabolic activity is based on the cell number.

Results: The aqueous extract effectively decreased number of HT29 cells (from 100 % in the control to 8 ± 7 % after 72 h with 20 g/l carob fibre). The effects were associated with dose and time. The reduction of metabolic activity was particularly apparent after 12 and 24 hours treatment (up to 32 % after 12 h with 2 g/l carob fibre), but was only temporary. In contrast, cell number of LT97 cultures was less impaired by the treatment with equal amounts of carob extract (70 ± 6 % of the medium control after 72 h with 20 g/l carob fibre) and metabolic activity correlated highly with cell number.

Conclusion: Carob fibre modulates the growth of both colon carcinoma and colon adenoma cells. The impact of the complex aqueous extract seems to depend on growth kinetics of the cell line. Highly proliferating carcinoma cells were more susceptible to the growth inhibitory properties of this plant extract, than cells with a lower cell turn over.

The effect of apple polyphenol extracts on proliferation of colon adenoma (LT97) and carcinoma (HT29) cells

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Introduction: Epidemiological findings suggest that plant foods decrease colorectal tumour risks. This could be due to a number of different phytoprotectants which act chemopreventive by inhibiting the growth of tumour cells. Although apples are rich in such phytochemicals (polyphenols), no attempt has been made to identify the relevant bioactive component(s).

Aim: We evaluated antiproliferative properties of apple polyphenols in colon adenoma (LT97) and carcinoma (HT29) cells to assess potential chemoprotective activities.

Material and Methods: We have used different apple polyphenol extracts "AE" (samples 1, 2 and 3) from different cultivars (2002-2004), which were characterized for their polyphenols and flavonoids by HPLC. LT97 and HT29 cells were first grown in 96-well plates for 72 and 48 h and then incubated with either medium alone (control) or apple extracts. The concentrations of AE were ranged from 8.5 – 85 µg (dry matter: equivalent to 5 – 50 µM of phloridzin). Cell growth was determined indirectly by measuring the total cell-DNA staining with the flouochrome 4', 6-Diamidino-2-phenylindole (DAPI) after 24, 48 and 72 h.

Results and Conclusions: All three AE contained high and comparable total amounts of polyphenols (478-533 mg/g). The comparison of the three AE samples revealed that sample 3 contains the highest amounts of quercetin derivatives and phloridzin (5.3 fold more than samples 1 and 2), probably due to the different qualities of the apple cultivars. Associated with this was the finding that sample 3 inhibited the growth of colon cells more than the other two samples. The LT97 adenoma and preneoplastic cells were more sensitive than the rapidly proliferating tumor-derived cells HT29. This suggests that AE contain polyphenols which suppress growth of adenoma and tumor cells, and thus may act chemoprotective, especially in the earlier stages of tumor development.

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The changes of the vitamin C concentration in the serum of patients with skin cancer

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Introduction

According to the opinions of many authors the chronic exposure to ultra-violet radiation constitutes a leading factor of skin carcinogenesis (Ananthaswamy et al., 1999; Andreasi et al., 1999; Armstrong and Kricger, 1996; Bloch and Gartner, 1999; Giacosa and Filiberti; 1996; Vahlquist, 1999). The photochemical reactions are, from the biological point of view, important phenomena caused by the absorption of the energy of the UV radiation. The energy of the photons of this radiation is so great that after their absorption there may happen a desintegration of the molecules (photolysis). Yet after the absorption of the UV photons most often an excitation occurs of atoms and molecules – the intensification of the free radicals (ROS) formation (Bloch and Gartner, 1999; Giacosa and Filiberti; 1996).

The antioxidant systemic mechanisms, including vitamin C (vit. C) take part in the struggle against free radicals. The strong reducing properties of the ascorbic acid decide of its antioxidant properties mainly expressed in the presence of O_2^{\bullet} , H_2^{\bullet} , H_2O_2 , HO^{\bullet} , $HOCL$, peroxide radicals and singlet oxygen (Bloch and Gartner, 1999; Frei et al., 1989; Halliwell, 1991; Henson et al., 1991). However, so far much less attention was brought to the disturbances of the ascorbic acid concentration associated with the carcinogenesis process in the skin. The existing thematic lacuna prompted our own studies, the purpose of which was to estimate the changes of the vitamin C concentration in the serum of patients with non-melanomatous skin cancers (NSC).

Materials and Methods

The study comprised 113 patients treated because of NMSC. The group consisted of 52 men and 61 women aged 32 to 84 years. In all of them the diagnosis of the

neoplasm was confirmed histopathologically. In 76 patients a baso-cellular cancer was diagnosed, in the remaining 37 – a spino-cellular cancer. The determinations were carried out of the vitamin C concentration in the serum in the group of patients with NSMC and the other two groups:

- a control group (CG) was composed of 70 patients with alopecia areata, lichen planus or with common warts;
- a reference group (RG) was made up of 36 blood donors.

In all of the patients the determinations of the vitamin C concentration in the serum were conducted with the chromatographic method (HPLC). The vitamin supply was standardized in all the persons analysed.

The range of the reference values of the vitamin C concentration in the serum amounting to 0.91 ± 0.18 mg/dl was established on the basis of the results obtained from the examination of 21 men and 15 women, a group of blood donors aged 19 to 56 years.

The statistical analysis

The statistical analysis of the results obtained was conducted using the SAS programme for IBM PC rel. 6.03. To estimate the statistical differences between the results having a normal distribution was applied t-Student's test, and for non-normal distribution Wilcoxon's test. All decisions were taken on the critical level $p < 0.05$.

Results

The vitamin C concentration in the serum of patients with NSV varied from 0.38 to 1.42 mg/dl, in the CG – from 0.52 to 1.24 mg/dl, whereas in the RG – from 0.80 to 1.22 mg/dl. The average vitamin concentrations for the respective groups are presented in table 1.

Statistically significant differences occurred in the average vitamin C concentration between the groups of patients studied. The lowest values of ascorbates concentration were observed in the patients with NSC, and the highest – in the RG.

The changes of ascorbic acid concentration in patients with NSC in relation to the location of the neoplasm are presented in table 2. The results testify that the lowest vitamin C average concentration is associated with the neoplasms developing on the face and neck skin, on the other hand the highest occurred in patients with NSC on

the trunk skin. In comparison with the CG, the average ascorbates concentration in the serum of patients with skin cancer of the face and neck as well as of the limbs was statistically significantly lower.

Table 1. Changes of the vitamin C concentration in the serum of patients with non-melanomatous skin cancers, in the control group and in the reference group.

Group studied	Vitamin C concentration in serum Arithmetic mean average \pm Standard deviation [mg/dl]
Patients with non-melanomatous skin cancer – 113 patients	0.61 \pm 0.21 ³
Control group – 70 patients with non neoplastic skin disorders	0.82 \pm 0.17 ^{1,4}
Reference group- 36 blood donors	0.91 \pm 0.18 ^{2,4}

¹ to ² - p<0.05; ³ to ⁴ - p<0.001

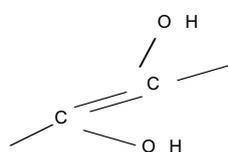
Table 2. Changes of the vitamin C concentration in the serum of patients with non-melanomateous skin cancers considering the location of the neoplasm.

Group studied	Vitamin C concentration in serum Arithmetic mean average \pm Standard deviation [mg/dl]		
	Location of the changes on the skin		
	Face and neck	Limbs	Trunk
Patients with non-melanomatous skin cancer	0.47 \pm 0.13 ³	0.54 \pm 0.18 ¹	0.82 \pm 0.24
Control group	0.79 \pm 0.16 ⁴	0.80 \pm 0.21 ²	0.87 \pm 0.27

¹ to ² - p<0.05; ³ to ⁴ - p<0.001

Discussion

One of the strongest antioxidants of the aqueous phase, forming the first defense line against the oxygenic radicals in this environment, is the L-ascorbic acid (vitamin C). It



is the gamma-lactone of the 2,3-dehydro-L-gulonic acid.

The most characteristic fragment of the L-ascorbic acid is the endiolo-group, the presence of which conditions the reducing properties as well as the acid character of that compound. The direct metabolite of the degradation of the

L-ascorbic acid, i.e. the dehydroascorbic acid has abilities to generate the redox

system similar to the L-ascorbic acid (Bloch and Gartner, 1999; Giacosa and Filiberti, 1996; Levine, 1986; Levine et al., 1993). Many authors have informed of the deficits of antioxidants in the neoplastic disease (Beissert and Schwarz, 1999; Berton et al., 1998; Block, 1991; Giacosa and Filiberti, 1996; Obermuller-Jevic et al., 1999; Traikovich, 1999). This phenomenon was confirmed also by our own studies showing that in patients with NSC the average ascorbates concentration in the serum was statistically significantly lower in comparison with the CG and the RG.

The relation of the changes of the vitamin C concentration to the location of the skin cancer allowed to confirm that the lowest average vitamin C concentration was associated with the neoplasm of the skin of the face and neck, whereas the highest occurred in patients with NSC of the trunk. In comparison to the CG, the average ascorbates concentration in the serum of patients with skin cancer of the face and neck as well as of the limbs was statistically significantly lower.

It is characteristic that the change of the vitamin C concentration were registered practically exclusively in patients with cancers developing on the skin on uncovered parts of the body, what may incline to link the generated vitamin C deficiencies with the mechanisms of the ultraviolet activity. This may have a link with the role, so far still unrecognised and unestimated, of the vitamin C in the process of induction, promotion and progression of the skin cancer of the body uncovered parts.

Conclusion

In patients with non-melanomatous skin cancers statistically significantly high vitamin C deficiencies in the serum are associated with the development of the skin cancer of the face, neck and limbs.

Summary

The studies include 113 patients (52 men and 61 women) aged 32 to 84 years, treated because of non-melanomatous skin cancer (NSC). In this group of patients, as well as in two others: a referenc group (RG) – blood donors, and a control group (CG) – patients with non-neoplastic skin disorders, the determinations were conducted of the vitamin C concentration in the blood serum with the chromatographic method. Following the studies carried out it was confirmed that in

patients with NSC a statistically significant decrease of the vitamin C concentration in the serum occurs in comparison with the RG and the CG. Yet these deficits mainly concern the cancer of the skin of the face and of the neck, as well as the cancer of the skin of the limbs.

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Excretion of polyphenols and metabolites in human urine following consumption of apple juice

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Introduction

In the German diet important sources of polyphenols are fruit juices. Apple juice is still the undisputed leader among the fruit juices with a per capita consumption of approximately 13 L / year (VdF 2003). The aim of the present study was to assess the urinary excretion of apple/ apple juice polyphenols (Figure 1) as well as of their potential metabolites in healthy humans.

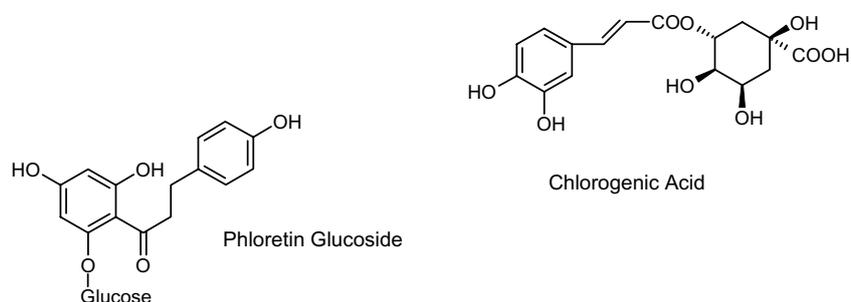


Figure 1. characteristic apple/ apple juice polyphenols.

Materials and Methods

After an overnight fast, 8 healthy non-smoking volunteers (8 females, 23-29 years old, BMI 22.9 ± 3.6 kg/m²) received a single oral dose of 700 ml of apple juice, produced from the cultivar *Weisser-Trierer-Weinapfel* (WTW; containing 1107 mg total phenolics, 330 mg chlorogenic acid, 69 mg phloridzin, 9 mg epicatechin, 7 mg phloretin xyloglucoside and 4 mg coumaroylquinic acid), or water as a polyphenol-

free control beverage (randomised cross-over design), together with a standardised breakfast. Urine samples were collected predose and in 4 intervals up to 24 h after dosing (0-2, 2-6, 6-10, and 10-24 h). During the experimental period, only the consumption of water and of two further standardised meals (lunch and dinner) was allowed.

Analytical Procedure: Total phenolics (photometrical; Singleton & Rossi, 1965), polyphenols and metabolites (HPLC-DAD and LC-ESI-MS/MS; Schieber et al., 2001), hippuric acid (HPLC-UV and LC-ESI-MS/MS; Kubota et al., 1988; Schieber et al. 2001), ascorbic acid and uric acid (HPLC-UV; Ross, 1994; Vazquez-Oderiz et al., 1994), were determined in the urinary samples of the volunteers.

Results

After *WTW* juice ingestion, the apple polyphenols were recovered predominantly as metabolites (glucuronides) in the volunteers' urine (Figure 2 and Table 1). Compared to the ingestion of water (control treatment), *WTW* juice consumption resulted in a significantly ($p < 0.05$) increased urinary excretion of total phenolics and hippuric acid (potential colon metabolite) within 24 h (Figure 3 and 4).

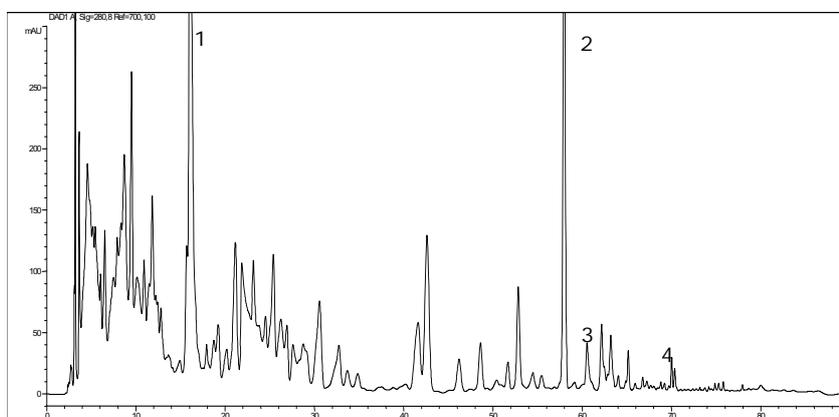


Figure 2. Representative HPLC chromatogram (HPLC-DAD at 280 nm) of human urine after *WTW* juice consumption. Peaks are as follows: (1) Hippuric acid, (2,3) Phloretin-monoglucuronides, and (4) Phloretin (sample preparation by SPE; identification by LC-ESI-MS/MS).

Table 1. Urinary excretion of polyphenols and metabolites (n = 8 adults).

identified compounds	control treatment (24 h-urine)	apple juice ¹ treatment (24 h-urine)
phloretin	nd	0.31 ± 0.15 mg ³
phloretin-monoglucuronides	nd	1.72 ± 0.71 mg ³
		→ 4.4 % compared to the ingested dose ²
chlorogenic acid caffeic acid vanillic acid	nd	in traces ³

¹cultivar: *Weisser-Trierer-Weinapfel*; data: mean ± SD; nd: not detectable; ²calculated as the ratio of amounts excreted (within 24 h) to amounts/dose ingested (as phloretin); ³identified by LC-ESI-MS/MS.

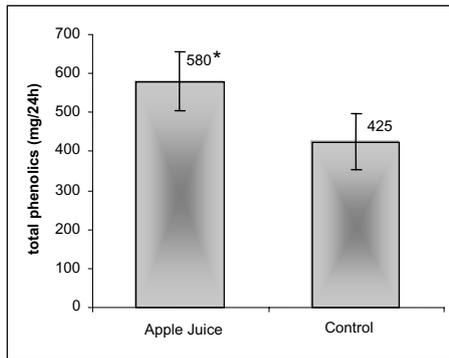
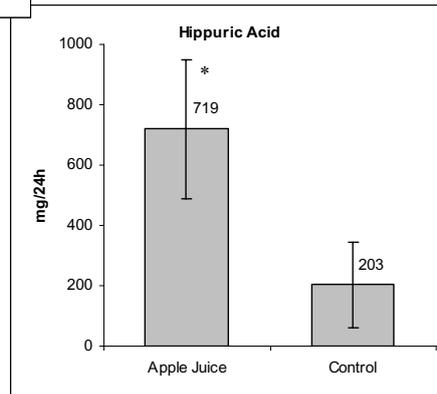


Figure 3. Total phenolics (apple juice vs. control; 24 h urine); data: mean ± SD; *p < 0.05, Wilcoxon-Signed-Rank test; values are corrected for ascorbic acid and uric acid. [+ 36 % compared to the control-treatment]

Figure 4. Hippuric acid (apple juice vs. control; 24 h urine); data: mean ± SD; *p < 0.05, Wilcoxon-Signed-Rank test. [+ 210 % compared to the control-treatment]



In conclusion, the metabolites (glucuronides and hippuric acid), and not the native apple polyphenols, may be responsible for at least some of the health effects attributed to apple/ apple juice consumption.

Summary

In Germany, apple juice is still the undisputed leader among the fruit juices with a per capita consumption of approximately 13 L / year. The aim of the present study was to assess the urinary excretion of apple/ apple juice polyphenols as well as of their potential metabolites in eight healthy humans. After a single oral dose of 700 mL of apple juice, produced from the cultivar *Weisser-Trierer-Weinapfel (WTW)*, the apple polyphenols were recovered predominantly as metabolites in the volunteers' urine. Compared to the ingestion of water (control treatment), *WTW* juice consumption resulted in a significantly increased urinary excretion of total phenolics and hippuric acid within 24 h. In conclusion, the metabolites (glucuronides and hippuric acid), and not the native apple polyphenols, may be responsible for at least some of the health effects attributed to apple/ apple juice consumption.

Acknowledgement

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Vitamin E and polyphenols do not prevent ochratoxin A-induced cytotoxicity in liver (HepG2) cells

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Introduction

Ochratoxin A (OTA), a mycotoxin mostly produced by *Aspergillus ochraceus* and *Penicillium verrucosum*, is a worldwide contaminant of food and feedstuff. It has been shown that oxidative damage contributes to the wide range of toxic effects of OTA (Rahimtula et al., 1988). Therefore, we examined the effects of α -tocopherol (α -TOC) and different polyphenols - catechin (CAT), daidzein (DAI), epicatechin (EC), epigallocatechin gallate (EGCG), genistein (GEN), and quercetin (QUE) - on OTA-induced cytotoxicity in HepG2 liver cells.

Materials and Methods

Cells of the human hepatoblastoma derived cell line HepG2 were cultured in RPMI media supplemented with 10% foetal calf serum, 2 mM glutamine, 100 IU/ml penicillin and 100 μ g/ml streptomycin under standard conditions. Confluent cells were harvested and seeded at 2×10^5 cells per well in 12-well plates.

In dose-response experiments, HepG2 cells were exposed to increasing concentrations of OTA ranging from 0-100 μ M for 24, 48, and 72 h. The cytotoxicity of OTA was measured by the neutral red (NR) assay (Valacchi et al., 2001). The antioxidant capacity of the test substances was determined with TEAC and DPPH assays (Re et al., 1999; Sanchez-Moreno et al., 2002).

Results

Incubation of HepG2 cells with increasing concentrations of OTA resulted in a dose- and time-dependent cytotoxicity (Figure 1).

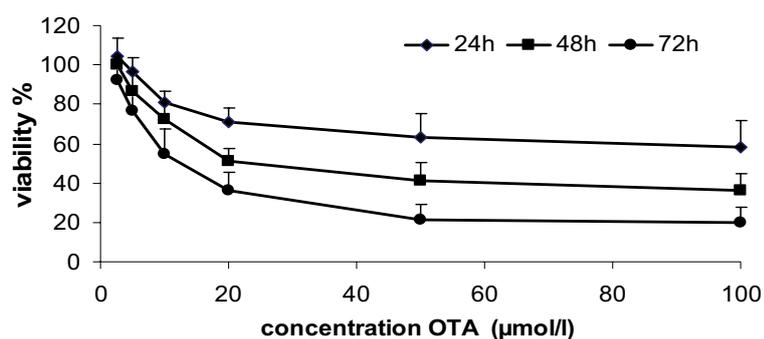


Figure 1: Viability of HepG2 cells after 24, 48, and 72 h of incubation with ochratoxin A at the given concentrations. Results are expressed as mean \pm SEM.

Regarding the TEAC and DPPH assays EGCG exhibited the highest antioxidant activity, followed by QUE, EC and CAT. The isoflavones showed the lowest antioxidant capacity (Figure 2A and 2B).

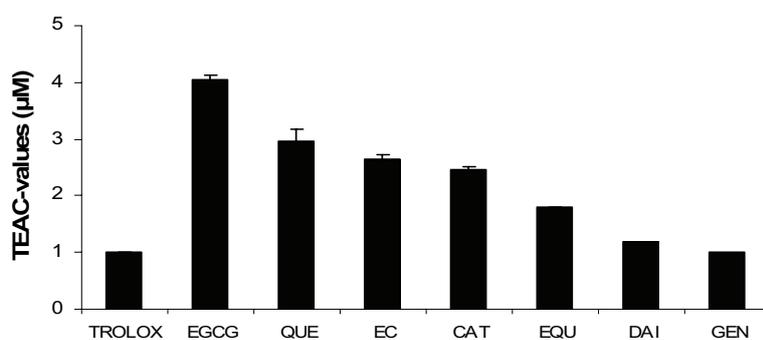


Figure 2 A: Antioxidant capacity of polyphenols as determined by the TEAC assay. Results are expressed as mean \pm SEM.

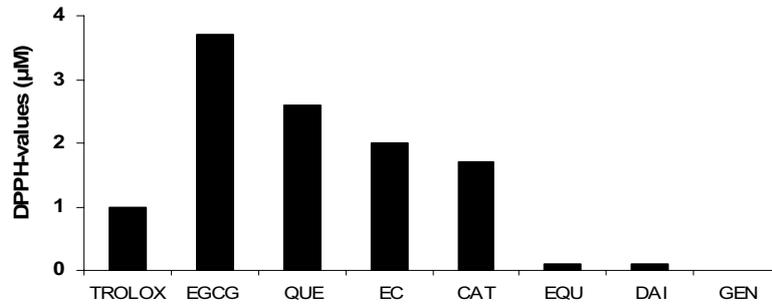


Figure 2B: Antioxidant capacity of polyphenols as determined by the DPPH assay. Results are expressed as mean \pm SEM.

Pre-incubation of HepG2 cells with α -TOC as well as with polyphenols did not significantly counteract OTA-induced cytotoxicity (Figure 3).

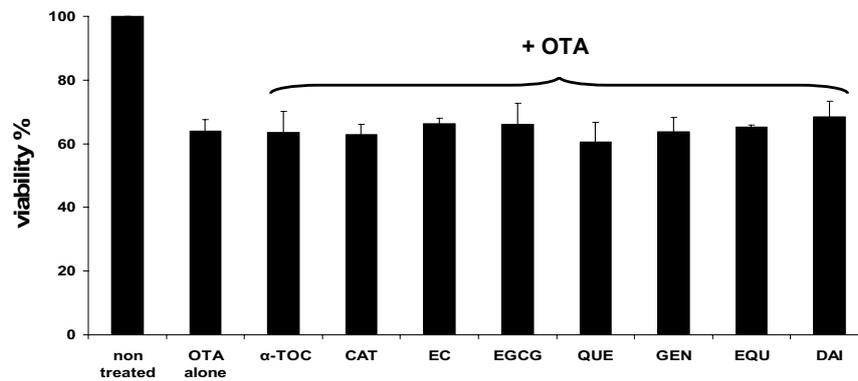


Figure 3: Viability of HepG2 cells after 48 h pre-incubation with α -TOC (100 μ M) and polyphenols (50 μ M) followed by OTA treatment (20 μ M, 48 h). Results are expressed as mean \pm SEM.

Discussion

In the present study, the half lethal OTA concentration (LC_{50}) in HepG2 cells was 35 μ M after 48 h incubation as measured by the neutral red assay. This value is similar to those reported by Baldi and coworkers (2004), who found in canine kidney cells (MDCK cells) a half lethal OTA concentration of 10-36 μ M.

Several studies propose the involvement of oxidative stress in the toxicity of OTA (Belmadani et al., 1999, Schaaf et al., 2002). However, our data indicate that neither α -TOC nor polyphenols, independent of their different antioxidant potency and solubility, had a protective effect on OTA-induced cell death.

It needs to be taken into account that other mechanisms than oxidative stress are also likely to be involved in the cytotoxicity of OTA, including inhibition of protein synthesis (Creppy et al., 1984), inhibition of various enzymes such as ATPase (Siraj et al., 1981) and succinate dehydrogenase (Wei et al., 1985) as well as the formation of DNA adducts (Faucet et al., 2004). Overall, present data demonstrate that vitamin E and polyphenols do not counteract OTA-induced cytotoxicity in liver HepG2 cells. These findings indicate that OTA may exert its toxic effects by affecting other hepatic mechanisms than those directly modulated by vitamin E and polyphenols.

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Risk Assessment of Vitamins and Minerals in Foods

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Introduction

Food supplements and foods fortified with vitamins and/or minerals have become increasingly available and popular on the European market. National regulations for the addition of micronutrients to foods vary widely within the European Community. First steps towards harmonisation of existing regulations were the adoption of the Directive 2002/46/EC on food supplements and the publication of a draft proposal for the regulation of the voluntary addition of vitamins and minerals to foods. Both regulations contain lists of the compounds of vitamins and minerals that may be added to supplements and foods. As some micronutrients may cause adverse effects if consumed in excessive amounts, values for maximum levels of vitamins and minerals added to those products are foreseen in the EU regulation. The objective of our risk-based assessment of these micronutrients was to develop a model to determine the level of each micronutrient that can be added safely to dietary supplements and/or foods per daily portion of consumption. The results were published in a two-volume report and are also available via Internet (Domke et al., 2004).

Methods and Results

The following four steps were applied in establishing maximum levels of vitamins and minerals for use in dietary supplements and fortified foods:

Step 1: Nutrient description, substance characterisation, knowledge of the metabolism, function, and where relevant, interaction with other nutrients and the Recommended Dietary Allowances (RDA) or Population Reference Intakes (PRI).

Step 2: referring to exposure estimates including representative food intake data or status, biomarkers, and identification of groups susceptible to sub-optimal intake/deficiency or excessive intake/toxicity.

Step 3: Hazard characterisation describes the potential hazard of the micronutrient and possible adverse effects in relation to the dose. Based on toxicological parameters like, for instance, NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level), tolerable upper intake levels (UL) have been derived by the EU Scientific Committee on Food (SCF) or the European Food Safety Authority (EFSA) or other bodies using uncertainty factors (UF). SCF understands the UL to be the highest level of daily nutrient intake from all sources that is very likely to pose no risk of adverse health effects on chronic intake by man (SCF, 2000).

Step 4: Risk characterisation has taken into account the conclusions from steps 1 to 3, including attendant uncertainties and lack of data.

Nutrients can be classified according to the risk of adverse effects through excessive intake (Tab. 1) and to the degree of risk of inadequate intake (Tab. 2).

Table 1. Risk of excessive intake

Categories	Criteria
High	Nutrients for which the margin between the RDA (or measured intake) and UL is low (factor ≤ 5) (e.g. vitamin A, D, copper, iron, zinc)
Moderate	Nutrients for which the UL is 5 to 100 times higher than the RDA (or actual intake) (e.g. vitamin E, K, C, B₆, magnesium, molybdenum)
Low	Nutrients for which a UL cannot be set as no adverse effects have been observed till now, even at intake 100 times the RDA (e.g. vitamin B₁, B₂, B₁₂, chromium)

Table 2. Risk of inadequate intake

Categories	Criteria
1	Risk of a clinically manifest deficiency or a store depletion in specific age groups, with specific physiological conditions/ with specific eating habits/ in specific regions (e.g. folate, iodine, vitamin D)
2	Uncertainty about the risk of a clinically manifest deficiency or a depletion of body stores because of the lack of or the questionable validity of biomarkers, inadequate food tables, or a lack of epidemiological studies (e.g. vitamin K, biotin, fluoride, zinc, selenium)
3	No indication of inadequate nutrient intake, or nutrient intake in the range of recommended intake (e.g. vitamin B₁, B₂, copper)
4	Indication of nutrient intake above recommended intake (e.g. vitamin B₆, B₁₂, sodium, chloride, phosphorus)

Derivation of maximum levels of micronutrients for addition to supplements and foods

The basic assumption is that the tolerable upper intake level of a vitamin or mineral (UL), derived by the EU Scientific Committee on Food (SCF) is already used up to a certain degree through the normal consumption of solid and liquid foods. The

resulting difference to the UL is the respective residual amount (R) of vitamin and/or mineral intake which may be provided by food supplements and fortified foods without exceeding the UL. In line with a precautionary approach, the highest percentile available from consumption studies (P 95 or P 97.5) was used as the value for Dietary Intake by Normal Food (DINF).

The risk model is based on the following formula and factors:

$$(1) \quad \mathbf{R = UL - DINF} \quad \text{e.g., folic acid} \quad \begin{aligned} R &= (1000^* - 0) \mu\text{g/day} \\ R &= 1000 \mu\text{g/day} \end{aligned}$$

* UL only applies to folic acid

- R** = Residual or maximum amount for safe addition to foods including dietary supplements
- UL** = Tolerable Upper Intake Level (SCF)
- DINF** = current estimated level of intake of the respective micronutrient from non-fortified food

The resulting figure (R) constitutes the amount available for addition to supplements (R_S) and fortified foods (R_F). The percentage of “R” to be allocated to supplements and fortified foods is selectable and may vary between 0 and 100%.

$$(2) \quad \mathbf{R = R_S + R_F} \quad \text{e.g., folic acid} \quad \begin{aligned} R (1000 \mu\text{g/day}) &= R_S + R_F \\ R &= (500 + 500) \mu\text{g/day} \\ \text{or } R &= (400 + 600) \mu\text{g/day} \end{aligned}$$

Considering that people might take more than one supplement or consume several portions of fortified foods per day, a Multi-Exposure Factor (MEF) has been introduced, and maximum levels (ML) for single portions of supplements or foods were calculated as follows:

$$(3) \quad \mathbf{ML_S} = \frac{\mathbf{R_S}}{\mathbf{MEF}} \quad \text{e.g., folic acid} \quad \mathbf{ML_S} = \frac{400 \mu\text{g/day}}{1} = 400 \mu\text{g/day}$$

$$(4) \quad \mathbf{ML_F} = \frac{\mathbf{R_F}}{\mathbf{MEF}} \quad \text{e.g., folic acid} \quad \mathbf{ML_F} = \frac{600 \mu\text{g/day}}{3} = 200 \mu\text{g/day}$$

- MEF** = Multi-Exposure Factor = estimated number of food supplements and fortified foods with the respective nutrient

Other nutrients for which this model was applicable are vitamin B₆, zinc and potassium. However, in the majority of nutrients it was not or not fully applicable or did not

lead to reasonable results. The available data – or more appropriately the sparse data – normally meant that nutrients had to be considered on an individual basis. The reasons, which restricted or ruled out the application of the method or did not lead, to reasonable results are the following:

SCF did not derive a UL due to lack of data, considerable degree of uncertainty or an insufficient scientific basis (e.g. β -carotene, vitamin C, manganese, chromium, phosphorus, iron, sodium, and chloride). This also refers to nutrients with a low toxicity and lack of systematic toxicological studies (e.g. vitamin B₁, B₂, B₁₂, K, pantothenic acid, biotin). Other reasons were that not enough data were available on the dietary intake (fluoride, selenium, and molybdenum) or that the intake via normal foods at the 95 or 97.5 percentile was above the UL (vitamin A, calcium). Depending on the desired level of protection, various options for setting maximum levels are outlined in the two-volume report and the respective advantages and disadvantages are discussed there in more detail (Domke et al., 2004).

Conclusion and Summary

For the first time, maximum levels of micronutrients for addition to both food supplements and fortified foods per daily portion of consumption have been derived based on risk assessment. As the proposed model is based solely on safety considerations, BfR strongly emphasises that nutritional aspects have to be taken into consideration as well when establishing maximum levels of micronutrients. Whenever the model is not applicable, the BfR recommends that maximum levels should be set on the basis of physiological and nutritional aspects.

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Vitamine und Kalzium als Zusatzstoffe in Milch (Kuh)
(*Vitamins and Calcium, as Additives in milk /cow/*)

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Introduction

In den Jahren 1998-2004 wurden im Rahmen des staatlichen Programms zur Prophylaxe Spurenelemente (I, Fe, F usw.), Mengenelemente und Vitamine von dem nationalen Zentrum für Ernährung die Bevölkerung in Georgien untersucht. Bei den Untersuchungen wurde festgestellt, dass es bei der Bevölkerung Georgiens bei Vitaminen (> 50%) und Ca (> 25 % und mehr) einen Mangel gibt. Die Stadtbevölkerung leidet im Vergleich zu den Dorfbewohnern an mehr Avitaminose-Krankheiten und die Entwicklung des relativen Risikos ist 2,5. Avitaminose, Rachitis, Atherosklerose, Osteoporose und andere Krankheiten sind bedingt durch den Vitamin- und Kalziummangel der Bevölkerung Georgiens. Besonders gefährdete Gruppen sind Kinder, Schwangere und Stillende.

Unvollwertige Ernährung ist der Grund der genannten Krankheiten bei der georgischen Bevölkerung (sowohl bei den Kindern als auch bei den Erwachsenen). Im Jahre 2005 wurde in Georgien ein Gesetz erlassen, das Lebensmittel mit Zusatzstoffen und Vitaminen erlaubt. Eine ausreichende Vitamin- und Kalziumzufuhr bildet somit die Basis der Prophylaxe gegen Avitaminose, Rachitis, Atherosklerose Osteoporose und andere Erkrankungen. Seit 6 Monaten wurde in Georgien mit Vitaminen und seit 3 Jahren mit Kalzium angereicherte Kuhmilch hergestellt und dies zu erschwinglichen Preisen für die Bevölkerung. Milch ist ein wichtiges Lebensmittel für die Gesundheit, besonderes für Kinder, Schwangere und Stillende. Der wichtigste Vorteil der Milch ist, dass sie für den menschlichen Organismus lebensnotwendige Nährstoffe wie Proteine, Fette, Kohlenhydrate, Vitamine und Mineralstoffe in einer sehr gut verfügbaren Form enthält. Bei der Pasteurisierung werden der Kuhmilch mit 2,5% Fettgehalt die Vitamine A, B1, B2, B3, B6, B12, Bc, E, D, PP, H und C hinzugefügt. Bei der Vitaminisierung der Kuhmilch ist der Fettgehalt 2,5%; die Dichte 1027, der Säuregrad 0 OT. In 100 Gramm Kuhmilch sind enthalten: Vitamin A 0,25 ME, D 0,25 DE, E 1,9 mg, B1 0,35 mg, B2 0,35 mg, B3 (Nikotinsäure) 1,75 mg, B6 0,4 mg, B12 0,75 mkg, Bc (Folsäure) 100 mkg, C 15,0 mg, PP 4,5 mg und H 50,0 mkg. Der frischen und pasteurisierten Milchkuh wird auch CaHPO₄ hinzugefügt. In

dieser mit Kalzium angereicherten Kuhmilch beträgt der Fettgehalt 0,5 %, die Dichte 1028 und der Säuregrad 19 OT. 100 Gramm dieser Kuhmilch enthalten 300 mg Kalzium. Die Kuhmilch besteht, im Gegensatz zu den anderen Lebensmitteln, aus Ergokalziperol, das Kalzitriol bildet. Es ist ein Teil von dem komplizierten kontrollierten System, das zusammen mit den anderen Hormonen für die Erhaltung einer normalen Kalziumkonzentration im Blut sorgt. Das Ziel der Untersuchungen war die Bewertung der Verwendbarkeit einer mit Kalzium und Vitaminen angereicherten Kuhmilch für die sekundäre Prophylaxe des Kalzium- und Vitaminmangels.

Material und Methoden

Im Jahre 2005 wurde von dem nationalen Zentrum für Ernährung 51 10-16 jährigen Mädchen und 40 25-50 Jährigen Frauen untersucht, die im Blut minimale Spuren des ionisierenden Kalziums von 1,00-bis 1,05 mmol/l (die Spuren von ionisierendem Kalzium liegen zwischen 1,00 bis 1,3) aufwiesen. Von 40 untersuchten Frauen waren 24 praktisch gesund und 16 litten unter verschiedenen Schilddrüsenerkrankungen (Knoten, endemischem Kropf, postperiodische - Hypothyreosis u.s.w), und haben im Rahmen einer Hormontherapie Levoxyn genommen.

33 Mädchen haben einen diffusen Kropf mit Schilddrüsenstand Ia und Ib gehabt und sie wurden mit Kalziumjodid (200mg) behandelt (im Rahmen des staatlichen Programms zur Prophylaxe mit Spurenelementen I, Fe, F usw.), Mengenelementen und Vitaminen von dem nationalen Zentrum für Ernährung wurden Krankheiten mit Jodmangel untersucht und behandelt). Von den untersuchten Personen war keiner übergewichtig oder wies keiner andere pathologische Zustände (intensive Malabsorption, Vitamin D Mangel u. s. w), auf, die eine normale Aneignung des Kalziums von dem Körper verhindern. Diese untersuchten Personen hat vor der Untersuchung unregelmäßig Milchprodukte und nicht mit Ca und Vitaminen angereicherte Kuhmilch zu sich genommen. Die Untersuchung hat 2 Monate gedauert. In dieser Zeit hat der Untersuchung gegen stand 2 Gläser Kuhmilch (ein Teil mit Ca, ein Teil mit Vitaminen angereicherte Kuhmilch und ein Teil unangereicherte Kuhmilch) zu sich genommen. Vor und nach der Untersuchung (2 Monate) wurde bei der untersuchten Personen die Konzentration des ionisierten Kalziums im Blut und des Vitamin D im Serum bestimmt und sie haben In dieser Zeit

ihr Ernährungsverhalten nicht geändert und die untersuchten Personen haben keine Präparate mit Ca, Vitamine D und andere Zusatzstoffe bekommen.

Tabelle 1: Die Zusammensetzung der unangereicherten und der mit Vitaminen angereicherten Kuhmilch

Zusammensetzung der unangereicherten Kuhmilch (In 100g Kuhmilch)			Zusammensetzung der mit Vitaminen (Fettgehalt 2,5%)angereicherten Kuhmilch (In 100g Kuhmilch)		
Vitamine					
A	mg	0,03	A	ME	0,25
D	mkg	0,05	D	ME	0,25
E	mg	0,09	E	mg	1,9
C	mg	1,5	C	mg	15,0
B1	mg	0,04	B1	mg	0,35
B2	mg	0,15	B2	mg	0,35
B3	mg	0,38	B3	mg	1,75
B6	mg	0,05	B6	mg	0,4
B12	mkg	0,4	B12	mkg	0,75
Bc	mkg	5	Bc	mkg	100
PP	mg	0,1	PP	mg	4,5
H	mkg	3,2	H	mkg	50,0

Tabelle 2: Zusammensetzung der unangereicherten Kuhmilch und angereicherten Kuhmilch mit Ca

Ca - Zusammensetzung der unangereicherten Kuhmilch (in 100g Kuhmilch)			Ca - Zusammensetzung der angereicherten Kuhmilch (in 100 g Kuhmilch und mit Fettgehalt 0,5%)		
Ca	mg	120	Ca	mg	300

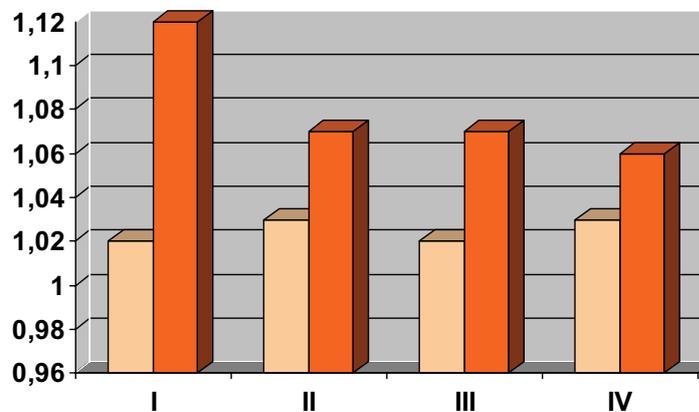
Jede Albumin Änderung verursacht eine Änderung der Gesamtkalziumkonzentration im Blut. Deswegen wurde jedem Teilnehmer die Konzentration des ionisierten Ca⁺⁺ im Blut (mit Easy Lyste Kalzium-Methode) und des Vitamin D (Immunoassay Methode) im Serum bestimmt.

Resultat und Zusammenfassung

Vor dem Beginn der Untersuchungen lagen die durchschnittlichen Parameter des ionisierten Ca⁺⁺ im Blut die untersuchten Personen zwischen 1,2 bis 1,03 mmol/l.

2 Monate danach war das ionisierte Kalzium im Blut am Stärksten bei den Gruppen V, I und VII (1,14mmol/l - 11,4%, 1,12mmol/l-8,9%, 1,11mmol/l-8,1%)erhöht. In den Gruppen III; II; VI; VIII war es (1,07 mmol/l - 4,7%, 1,07mmol/l - 3,7%, 1,06mmol/l - 3,8%, 1,1,06mmol/l) um 3,8% gestiegen. Am Wenigsten ist es bei der Gruppe IV – 1,06mmol/l – um 2,8% angestiegen.

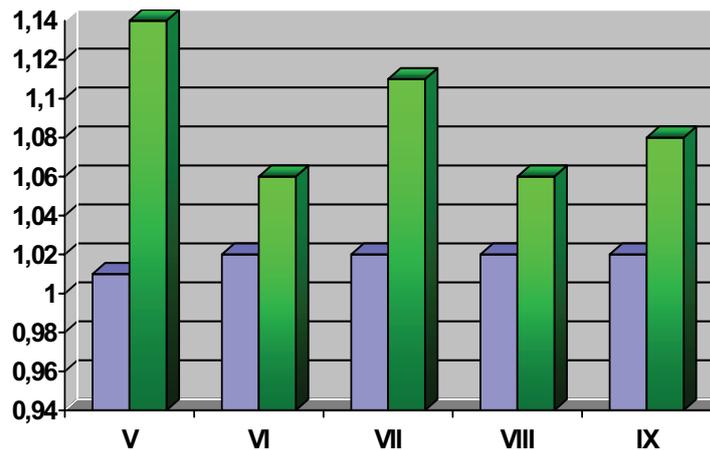
Abbildung 1. Das durchschnittliche Merkmal bei den Gruppen I; II; III; IV der jonisierten Kalziumkonzentration (mmol/l) im Blut 2 Monate vor und nach der Untersuchung



Die geringste Erhöhung der ionisierten Ca^{++} Konzentration bei der Gruppe II kann man damit erklären, dass das Trinken von normaler Milch für 2 Monate für die Prophylaxe der Hypokalziämie nicht ausreichend ist. Die geringste Erhöhung der ionisierten Ca^{++} Konzentration erklärt man bei den Gruppen III und IV damit, dass eine Einnahme einer großen Dosis Kalzium bei der Langzeit Therapie mit Thyroidhormonen notwendig ist /Abbildung 1/.

Die relativ starke Erhöhung der ionisierten Ca^{++} Konzentration bei den Gruppen V und VII, im Vergleich zu den Gruppen VI und VIII (die Kindern nehmen Ca leichter auf ,als Erwachsene) bestätigt, noch mal die Notwendigkeit der Einnahme mit Ca angereicherte Kuhmilch von Kindern. (Abbildung 2)

Abbildung 2. Das durchschnittliche Merkmal bei den Gruppen V, VI, VII und VIII der ionisierte Kalziumkonzentration (mmol/l) im Blut 2 Monate vor und nach der Untersuchung.



Bei der Gruppe IX, wo die praktisch gesunden Mädchen die vitaminisierte Kuhmilch bekommen haben, ist die ionisierte Ca⁺⁺ Konzentration im Blut im Vergleich zu der Gruppe VI, wo die Probanden die unangereicherte Kuhmilch bekommen haben um 5,6% gestiegen. Die Konzentration von Vitamin D ist in der Gruppe IX um 7,6% gestiegen, im Vergleich zu der Gruppe VI, die unangereicherte Kuhmilch bekommen hat und Vitamin D Konzentration im Blut durchschnittlich um 2,3% gestiegen.

Zusammenfassung

Durch die Untersuchungen bei den Mädchen und Frauen wurde festgestellt, dass mit Ca und Vitaminen angereicherte Kuhmilch auf die sekundäre Prophylaxe positive Auswirkungen hat.

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Stability of RRR- α -Tocopheryl-Acetate in premixes and feed processing

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ABSTRACT

A comparative study was carried out to assess the stability of natural (*RRR- α -Tocopherol*) and synthetic (*all-rac- α -Tocopherol*) vitamin E sources in acetate form. Two premixes (standard; aggressive with elevated levels of Cu, choline, salt) were prepared with the two vitamin E sources. The standard premix was added to a piglet feed in mash form, which was pelleted (75°C) and extruded (90, 100, 110°C). During 12 months, storage stability at ambient (20°C) and elevated (40°C) temperature was tested for the following treatments: standard premix, aggressive premix, mash, pelleted feed and extruded feed.

Manufacturing stability: The vitamin E recovery after pelleting was 100%. The extrusion process, however, reduced vitamin E recovery by about 40%. Extrusion temperature did not impact on the extent of *α -tocopherol* losses in the extrusion process. No differences in processing stability have been observed between natural and synthetic vitamin E preparations.

Storage stability: Vitamin E in acetate form was stable in premixes during the tested period of 12 months. No differences were observed in storage stability between natural and synthetic vitamin E sources during the 12-month period. Premix type (standard, aggressive) had no effect on storage stability. Storage temperature did not affect vitamin E recovery in premixes during 12-month storage. However, it seems that high storage temperature reduced vitamin E recovery compared to low temperature storage in mash, pelleted and extruded feeds after 6 months storage. In conclusion, the processing and storage stability in premixes and feeds supplemented with natural vitamin E (*RRR- α -tocopherol*) is the same as with synthetic vitamin E (*all-rac- α -tocopherol*), when the products are in acetate form. Vitamin E is stable in premixes during 12-month storage. When stored at 20°C, vitamin E is stable in mash feed up to 9 months and up to 6 months in pelleted and extruded feeds.

INTRODUCTION

Natural-source vitamin E is derived from vegetable oils and exists in the form of *RRR- α -tocopherol* (formerly *d- α -tocopherol*). Synthetic vitamin E consists of equal amounts of eight isomers known as *all-rac- α -tocopherol* (formerly *dl- α -tocopherol*). It has long been recognized that natural vitamin E has higher bio-potency in humans and many animal species than synthetic vitamin E. In 2000, Food and Nutrition Board

(FNB), U.S. National Academy of Sciences has officially defined that 1 mg *RRR- α -tocopheryl acetate* equals 2 I.U.

Feed grade natural vitamin E is commercially available since 2004. Because of the high bio-efficiency of natural source vitamin E and with the upcoming withdrawal of antibiotic growth promoters, feed producers are paying more attention to natural source vitamin E. The processing and storage stability of natural source vitamin E, however, is of concern for premix companies and feed producers alike. Up to date, no direct comparison has been made on stability between feed grade natural and synthetic source vitamin E. The objective of this study was to compare the stability of natural source vitamin E, i.e. *RRR- α -tocopheryl acetate*, with synthetic vitamin E, i.e. *all-rac- α -tocopheryl acetate* in feed manufacturing and during storage.

MATERIAL and METHODS

Two premixes were tested in this study. Premix I was a standard premix with normal level of Cu and choline and premix II contained elevated levels of Cu, choline and additional salt. The composition of the two premixes is given in Table 1.

Table 1. Composition (g/kg) of premixes

	Premix I (Syn/Nat)	Premix II (Syn/Nat)
Wheat feed flour	337.2 / 321	-
<i>Vit E (Syn / Nat*)</i>	<i>64.9 / 81.1</i>	<i>64.9 / 81.1</i>
Antioxidant	5	-
Vit A + D3	3.2	3.2
Vit B1, B2, B6, B12	7.05	7.05
Ca-Pant , Nicotinic acid	8.3	8.3
K3 , Folic acid, Biotin	1.75	1.75
Choline chloride	41.7	83.4
Salt	-	483.8 / 467.6
Ca- carbonate	250	-
Fe- sulfate	100	166.7
Cu – sulfate	128	128
Mn + Zn- oxide	41.3	41.3
J + Co- prep.	5.6	5.6
Na- selenite	6	6

* Synthetic vitamin E 50%; Natural source vitamin E 40%

Premix I was used to make piglet feed in mash form that was supplemented with 175 mg/kg of natural or synthetic vitamin E, respectively. The mash feed was pelleted at 75-78°C or extruded at 90, 100 and 110°C, respectively. Three samples were taken

as parallel samples (50g each) at 0, ½, 1, 2, 3, 6, 9 and 12 months after processing and stored in closed plastic bags in a climatic chamber at 20 and 40°C, respectively. For piglet feed, samples (4 x 50 g) were taken from the mash before pelleting or extrusion. The sample bags were held under mechanical pressure (0.1 kg/cm²) to simulate pallet storage conditions. Vitamin E was analysed as *α-tocopheryl acetate* using capillary gas chromatography-method without derivatisation. The analytical tolerance level for the given concentration in the premixes is ± 10% relative and ± 20% relative for mash and processed feeds (VDLUFA 1997).

RESULTS

Processing stability

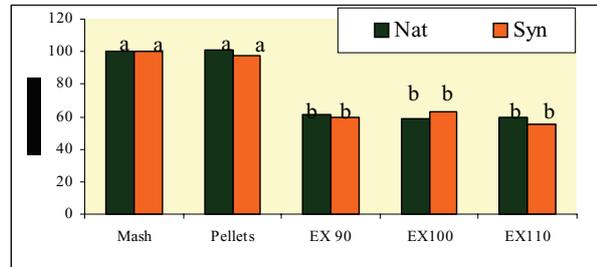


Figure 1. Recovery of vitamin E from synthetic and natural source in mash, pelleted and extruded feeds after processing (a,b-columns with different letters differ significantly; P<0.05)

No differences were observed for processing stability between natural and synthetic vitamin E, indicating that the stability of natural vitamin E is the same as synthetic vitamin E.

Vitamin E recovery after processing was 100% in both mash and pelleted feed, indicating that the pelleting process did not reduce vitamin E activities in the feed. The extrusion process, however, reduced vitamin E activity to an average recovery level of 60%. Extrusion temperature had no effect on vitamin E recovery (Figure 1).

Storage stability

Premixes

In premixes, no significant differences in storage stability between natural and synthetic vitamin E occurred during 12 months storage. Furthermore, it was observed that neither storage temperature, nor premix type effected on storage stability (Figure 2).

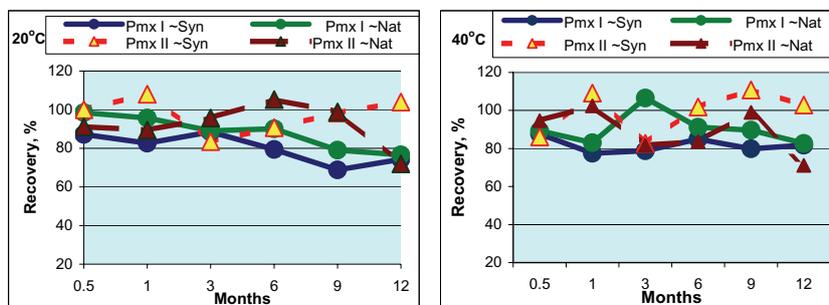


Figure 2. Recovery of vitamin E from synthetic and natural source in premixes during 12 months storage at 20°C (left) and 40°C (right)

Mash feed

No significant differences were observed in storage stability between natural and synthetic source vitamin E in mash feed during 12 months storage. As shown in Figure 3, the relative recovery of vitamin E after 9 months storage is >90% when stored at 20°, indicating that vitamin E is stable up to 9 months in mash feed. Apparently, vitamin E activity is reduced after 9-month storage and this is more pronounced at storage temperature of 40°C (Figure 3).

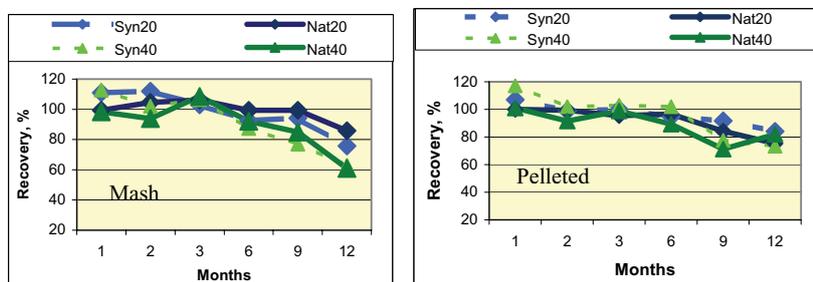


Figure 3. Recovery of vitamin E from synthetic and natural source in mash feed (left) and pelleted feed (right), during 12 months storage at 20°C and 40°C

Pelleted feed

Up to 12 months, no differences in storage stability between natural and synthetic source vitamin E were observed in pelleted feed (Fig. 3). When stored at 20°C, the mean value of vitamin E percentage recovery was around 80% at 9-month storage. When stored at 40°C, numerically higher losses occurred. Based on the results it can be safely concluded that vitamin E is stable in pelleted feeds up to 6 months, when stored at 20°C.

Extruded feed

Extruded feeds showed a numerical decline over the tested storage period; however, the 12-month storage stability in extruded feed was not affected by vitamin E sources. Storage temperature had no effect on vitamin E recovery till 6 months, but after 9 months, the vitamin E recovery seems to be lower at high storage temperature compared to low storage temperature (Figure 4). The variation in vitamin E analyses did not allow showing significant differences between the different time points. However, it can be safely concluded that vitamin E is stable in extruded feeds up to 6 months, when stored at 20°C.

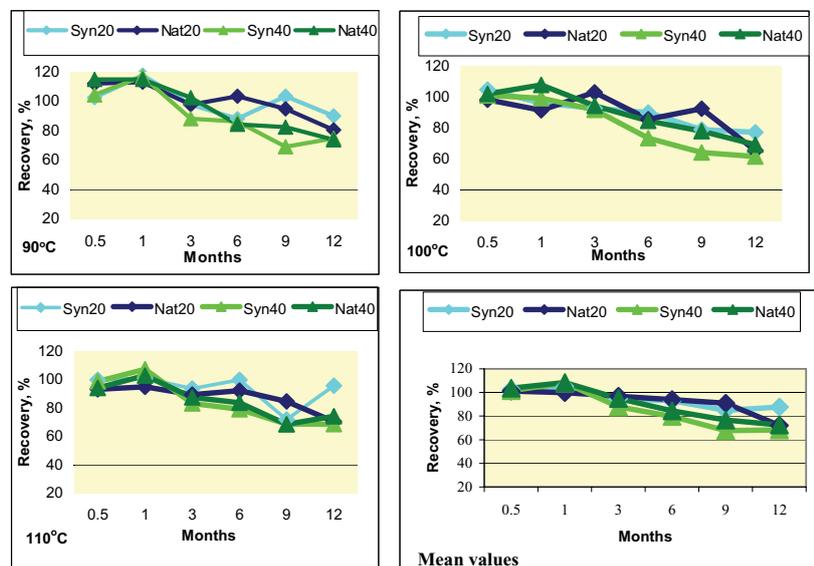


Figure 4. Recovery of vitamin E from synthetic and natural source in feeds extruded at different temperatures and mean values, during 12 months storage at 20°C and 40°C

Mean values

The mean values of vitamin E recovery for mash, pelleted and extruded feeds are presented in table 2. When stored at 20°C, the average vitamin E recovery, independent on storage temperature and treatment, was above 90% up to 6-month storage, above 85% up to 9-month storage and above 80% up to 12-month storage, respectively. However, due to individual values for some treatments that were below 80% recovery, a general recommendation for 9-month storage stability of vitamin E in pelleted and extruded feeds cannot be given. The mean value for all treatments, disregarding storage temperature showed no difference in storage stability between natural and synthetic vitamin E. Apparently, high storage temperature (40°C) reduced

vitamin E activity after 6-month storage more than storage at 20°C. To evaluate the losses of vitamin E activities, a linear regression was applied to determine percentage loss per month. Since in mash and pelleted feed the losses from 1 – 6 months were only minor, the regression was calculated for the 6 – 12 months period; whereas in extruded feeds the loss pattern seemed rather consistent, starting from the beginning of the storage test, the regression was calculated for the entire storage period. Over the storage period of 6 – 12 months, the monthly loss was estimated with about 3% for pelleted feed, independent on the storage temperature and 4.6% for mash feed, stored at 40°C. Extruded feeds exhibited a monthly loss rate of <2% and 3.3% when stored at 20° and 40°C, respectively, during 12 month storage. This confirms that high storage temperature (40°C) reduced vitamin E activity more than 20°C storage after 6 month.

Table 2. Mean values of vitamin E recovery (%) in mash, pelleted and extruded feed during 12 months storage at 20°C and 40°C

Storage Month	20°C			40°C		
	Mash	Pelleted ¹⁾	Extruded ²⁾	Mash ¹⁾	Pelleted ¹⁾	Extruded ²⁾
1	105.2	103.5	102.4	105.5	109	108.2
3	104.6	97.8	95.8	107.5	101.0	91.3
6	96.0	95.7	93.4	89.7	95.7	82.1
9	96.8	88	87.9	81.0	74.4	72.1
12	80.8	79.7	80.0	61.9	77.7	70.5
%	n.d.	-2.67	-1.88	-4.6	-3	-3.31
R ²		0.999	0.97	0.96	0.62	0.89

¹⁾ From 6 – 12 months of storage ²⁾ From 1 – 12 months of storage

CONCLUSIONS

No significant differences between *all-rac* and *RRR alpha tocopheryl acetate* in terms of processing and storage stability were observed. Apparently aggressive premix did not cause higher losses of vitamin E. Pelleting at 75°C did not trigger any losses in dietary vitamin E, whereas the extrusion process caused significant losses, independent on the tested extrusion temperatures. In premixes, vitamin E was stable during the 12-month test. When stored at 20°C, vitamin E is stable in mash feed up to 9 months and up to 6 months in pelleted and extruded feeds. Storage temperature did not impact on loss pattern up to 6-month storage; however, high storage temperature apparently reduced vitamin E recovery at and after 9 months.

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Bioavailability of flavonoids and other antioxidants in microwave-vacuum-dried strawberries

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Introduction

Strawberries are one of the most often consumed fruits in Germany (4.3 kg per person per year). They are consumed as fresh fruits as well as in a processed form. The interest in fruits and vegetables is increasing, due to their positive effects on health and well-being. Thus, research especially puts its focus on flavonoids and other antioxidant compounds having a health benefit. Strawberries are rich in phenolic compounds, e.g. flavonols and anthocyanins. As fresh fruits are not available the whole year, most of the time processed products are consumed. The aim of this study was to investigate the intestinal absorption of vitamin C, total phenolics and the antioxidant capacity after ingestion of microwave-vacuum-dried (MVD) strawberries compared to raw strawberries. Prior to this investigation, the MVD-process was optimised to decrease the losses of vitamin C and polyphenolics.

Material and Methods

After a one week wash-out-phase, eight healthy adults consumed 600 g raw strawberries and 50 g MVD strawberries using a cross-over design. Blood samples were taken prior to the investigation, 30/60/90/120/180/240/300 and 360 min after ingestion of strawberries. Urine samples were collected during 24 hours (0-3 h/3-6 h/6-9 h/9-24 h) after consumption of the strawberries. Vitamin C was determined photometrically after oxidation to dehydroascorbic acid and reaction with 2,4-dinitrophenylhydrazine (Speitling et al. 1992). The polyphenolics were assessed photometrically by using the Folin-Ciocalteu method (Singleton and Rossi 1965). Antioxidant capacity was determined by using three different assay systems (Ferric Reducing Antioxidant Power (FRAP), Photochemiluminescence (PCL), Trolox Equivalent Antioxidant Capacity (TEAC) (Schlesier et al 2002). Uric acid was measured photometrically by using a commercially available kit.

Results and Discussion

Uric acid is a non-enzymatic antioxidant in the organism. Therefore it was measured in order to exclude any interactions with other antioxidants. Uric acid levels in plasma and urine remained stable during the whole intervention. Data of the TEAC and FRAP test are similar to the results of the PCL assay.

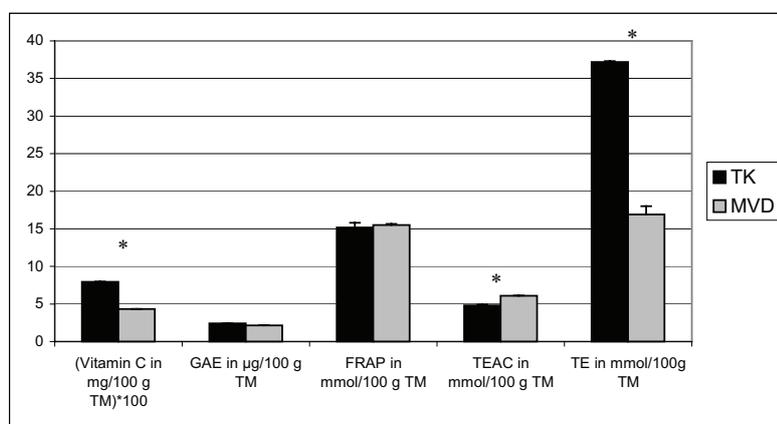


Figure 1: Contents of vitamin C, total phenolics (expressed as GAE) and antioxidant capacity in frozen (TK) strawberries compared to dried fruits (MVD), * significantly ($p < 0.05$) different values

Figure 1 shows significant differences between both kinds of strawberries for the contents of vitamin C and the antioxidant capacity values determined in the TEAC assay and the PCL assay. Contents of polyphenolics and FRAP values were similar ($p > 0.05$) for both strawberry types.

Vitamin C levels seem to decrease more slowly in both, plasma and urine samples (**Figure 2**) after ingestion of MVD fruits. Maximum values in blood samples of the MVD-group are also reached delayed (data of plasma not shown).

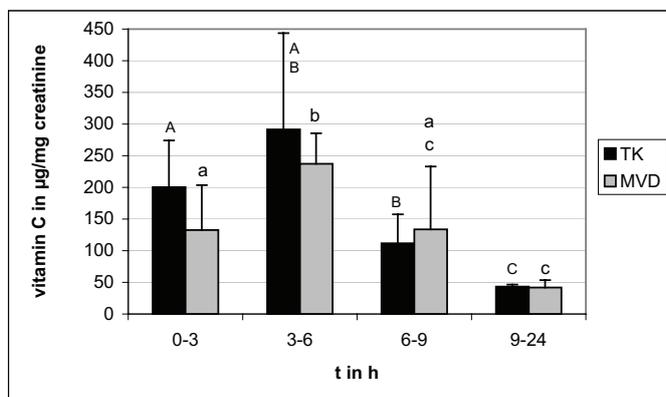


Figure 2: Contents of vitamin C in urine samples after strawberry consumption, different letters within strawberry type mark significant differences ($p < 0.05$)

Urine excretion differs between the two groups although the comparison of polyphenolics in strawberries does not show any significant difference. Levels of polyphenolics in the MVD-group remain almost stable during 9 hours after ingestion of fruits (**Figure 3**).

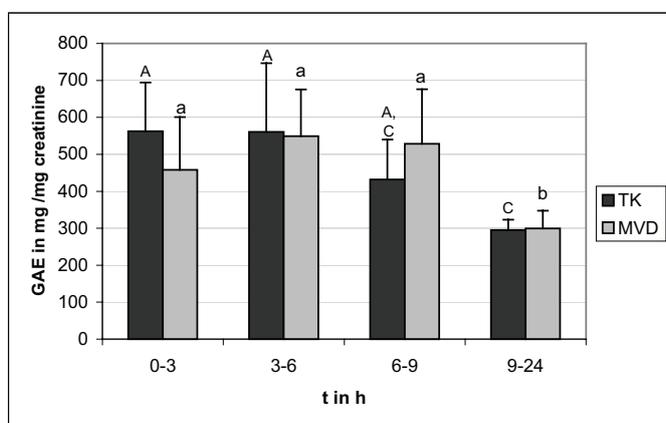


Figure 3: Contents of total phenolics (GAE) in urine samples after strawberry consumption, different letters within strawberry type mark significant differences ($p < 0.05$)

The PCL assay showed significant differences between the two types of strawberries. However, these differences were not found after ingestion of both kinds of fruits (**Figure 4**). The results confirm the delayed maximum after ingestion of MVD-strawberries.

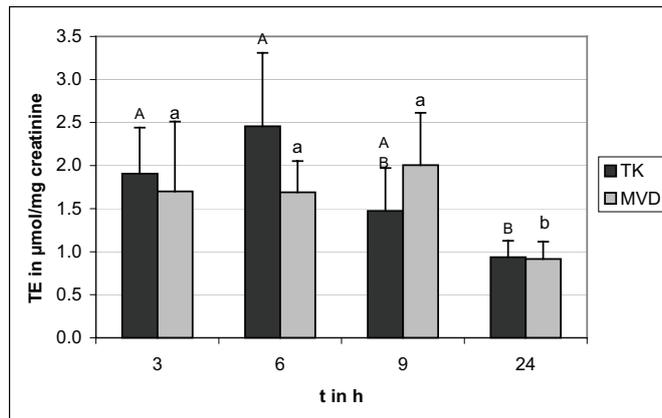


Figure 4: Antioxidant capacity expressed as of Trolox equivalents (TE) in urine samples after strawberry consumption (PCL assay), different letters within strawberry type mark significant differences ($p < 0.05$)

In plasma samples all investigated parameters were lower in the MVD-group compared to the other group. Maximum values were reached later after consumption of MVD strawberries compared to the fresh fruits. Excretion of all parameters investigated was also more slowly after ingestion of MVD-strawberries. Thus, the protective ingredients seem to remain longer in the organism after consumption of MVD-fruits. Thus, MVD-strawberries are a healthy snack to enrich the daily diet.

Acknowledgements

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Vitamin E and A concentrations trace element levels, and some antioxidant parameters in the blood of bladder cancer patients.

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It is generally believed that processes involving generation and promotion of reactive oxygen species and/or their metabolites play an important role in the initiation and development of numerous pathologic conditions including cancer. It is currently believed that antioxidants may directly reduce concentration of oxygen and its active forms (superoxide radical anion, hydrogen peroxide), bind metal ions, scavenge free radicals capable of initiating chain reactions with organic compounds. Enzymatic antioxidants include glutathione peroxidases (GPx), superoxide dismutase (SOD), glutathione S-transferase, and ceruloplasmin (Cp). Non-enzymatic antioxidants comprise tocopherols (vitamin E), β -carotene (provitamin A), ascorbic acid (vitamin C), lycopene, glutathione, uric acid, and bilirubin. Microelements (selenium, zinc and copper) associated with an active site or occurring in antioxidative enzymes as structural elements also play an important role.

The aim of the study was to determine vitamin E, A and β -carotene concentrations, selenium, zinc and copper levels and some antioxidant parameters (GPx, SOD, Cp activities, total antioxidant capacity (TAC)) in the blood of bladder cancer patients. The study covered 65 bladder cancer patients (men only) and 157 appropriate controls. The results of the study indicated statistically lower vitamin A and E concentrations in cancer patients ($p < 0.001$) as compared to controls. Copper level was significantly elevated ($p < 0.0001$) and was found to be 1.24 ± 0.28 mg/l in cancer patients vs. 1.00 ± 0.18 mg/l in controls. We did not find any differences between selenium and zinc concentrations in cancer subjects as compared to controls. Glutathione peroxidase activity in red blood cells of bladder cancer patients was found to be 22.0 ± 5.3 u/g Hb. This value was significantly higher than in healthy controls (17.3 ± 3.9 u/g Hb; $p < 0.0001$). Activities of SOD in erythrocytes ($p < 0.001$) and Cp in plasma (NS) as well as TAC concentration ($p < 0.0001$) were significantly higher in cancer patients. Statistically significant linear correlations between selected parameters determined were found. Further and more thorough research are needed to comprehend the relationship among the incidence of bladder cancer and the involvement of antioxidants in this process.

Dietary ascorbic acid supplementation: the good and the bad

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Introduction

Ascorbic acid is considered the most important antioxidative component of animal diet. It takes part in redox and hydroxylation reactions, it fulfils the different functions. Increased dietary supplementation with ascorbic acid is shown to be beneficial in many cases, including immunostimulation, prevention of a decrease in productive performance in various stress conditions. Several experimental studies have shown that ascorbic acid ingestion alters the prooxidant-antioxidant balance in the organism. The use of ascorbic acid as antioxidant has been advocated because of its ability to scavenge reactive oxygen species, although its potential for prooxidant activity in the presence of transition metal ions has also been recognized (Gille et al., 1995; Lee et al., 2001). Depend on doses ascorbic acid has dual effect on oxidation of low density lipoprotein cholesterol (Chen et al., 2002).

The aim of the present study was to investigate how L-ascorbic acid dietary loading can influence on antioxidant and immune status of experimental chickens.

Materials and methods

Five groups of one to 30-day-old male Lohmann Brown chicks were fed the diets differed only by L-ascorbic acid supplements (0, 100, 500, 1000 and 2000 mg/kg). At the end of the experiment the birds were weighted and sacrificed. To assess the metabolic and immune changes induced by L-ascorbic acid the next indices were studied: in blood – hematological parameters; in blood serum – creatinine and uric acid content, lysozyme and circulating immune complexes (CIC) level (Vasilyeva et al., 2001); in liver – malondialdehyde (MDA), glutathioneperoxidase (GSH-Px) activity, glutathione (GSH) level, cadmium (Cd) concentration (Apsite et al, 1999); in immunocompetent organs – T and B immunocyte number. Permeability of small intestine epithelium was evaluated by glycine accumulation *in vitro*. Statistical analysis was performed by paired samples test using program SPSS (Bühl et al., 2002).

Results

It is known that the low value of cadmium is present in poultry food due to environmental contamination. Our study indicated that ascorbic acid had effect on the accumulation of cadmium by liver (Table 1). The concentration of cadmium in liver increased ($p < 0.001$) when ascorbic acid was added to the diet (500, 1000, 2000 mg/kg). The results of investigation showed that high concentration of ascorbic acid (1000 and 2000 mg/kg) significantly diminished the activity of GSH-Px and the level of GSH, and caused the increase of MDA formation in the liver.

Table 1 Change in hepatic Cd accumulation, GSH level, GSH-Px activities and MDA formation in chicks received diets with different ascorbic acid level (mean \pm SE)

Group	Cd, $\mu\text{g}\cdot\text{g}^{-1}$	GSH, $\mu\text{mol}\cdot\text{g}^{-1}$	GSH-Px, $\mu\text{molGSH}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$	MDA, $\mu\text{mol}\cdot\text{g}^{-1}$
Control	0.22 \pm 0.02	4.83 \pm 0.14	4.46 \pm 0.32	29.56 \pm 0.84
+ 100 AA	0.26 \pm 0.03	4.92 \pm 0.18	4.49 \pm 0.27	32.05 \pm 0.70
+ 500 AA	0.38 \pm 0.04*	4.17 \pm 0.09*	4.37 \pm 0.11	31.51 \pm 1.73
+ 1000 AA	0.40 \pm 0.03*	3.91 \pm 0.12**	3.30 \pm 0.07**	42.17 \pm 1.87*
+ 2000 AA	0.46 \pm 0.03*	3.75 \pm 0.16 **	3.20 \pm 0.06**	34.68 \pm 0.73**

Different from the control group: * $p < 0.001$, ** $p < 0.02$

In blood serum of chickens received diets enriched by ascorbic acid (100, 500 mg/kg) the reduction of uric acid ($p > 0.05$) and creatinine ($p < 0.001$) concentration was observed (Table 2). Blood serum levels of uric acid and creatinine were significantly higher in the animals fed the high (1000, 2000 mg/kg) compared with the low dose of ascorbic acid.

Table 2 Effect of ascorbic acid supplementation on the protein catabolism indices in blood serum of chicks (mean \pm SE)

Group	Uric acid, mg %	Creatinine, $\mu\text{mol}\cdot\text{l}^{-1}$
Control	1.58 \pm 0.14	96.29 \pm 1.73
+ 100 AA	1.40 \pm 0.04	81.60 \pm 0.38*
+ 500 AA	1.41 \pm 0.08	86.76 \pm 0.18*
+ 1000 AA	2.01 \pm 0.16	119.51 \pm 3.38*
+ 2000 AA	2.11 \pm 0.09**	126.59 \pm 1.65*

Different from the control group: * $p < 0.001$, ** $p < 0.02$

Table 3 Permeability of small intestine epithelium, accumulation of glycine *in vitro* (% of control values)

Group				
Control	+ 100 AA	+ 500 AA	+ 1000 AA	+ 2000 AA
100	111.2	108	118*	125**

Different from the control group: * $p < 0.01$, ** $p < 0.001$

A marked increase in permeability of small intestine epithelium was observed in chicks received diets with high level (1000 and 2000 mg/kg) of ascorbic acid by 18 and 25% respectively (Table 3).

In the Tab. 4 the parameters of innate and specific immunity of chicks are represented. Lysozyme activity of blood serum in chicks fed diets enriched by ascorbic acid was reduced compared with the control. The most influence of ascorbic acid on this index was shown in +2000 AA group (5.8 times less than in control). The same direction of ascorbic acid effect on CIC was observed. Compared with the control values, the count of T- and B(C3)-lymphocytes in immunocompetent organs of +100 AA and +500 AA group chickens increased by 28.3% and 42.3% respectively. When high supplements of ascorbic acid (1000, 2000 mg/kg) were added to the diet these parameters are not distinguished from control. Moreover it was stated the increase of phagocytic activity of blood neutrophils by 31.6% in chicks fed the diets enriched by ascorbic acid till 500 mg/kg.

Table 4 Ascorbic acid-induced immunological changes in chickens (mean \pm SE)

Group	Lysozyme activity of blood serum, $\mu\text{g}\cdot\text{ml}^{-1}$	Circulating immune complexes (CIC) of blood serum, extinction units $\cdot 100$	Relative count of lymphocytes	
			Thymus, T-cells, %	Bursa of Fabricius, B(C3)-cells, %
Control	10.75 \pm 0.25	4.13 \pm 1.27	46.0 \pm 2.0	26.0 \pm 2.0
+ 100 AA	6.36 \pm 1.42*	4.20 \pm 1.82	58.0 \pm 3.0*	37.0 \pm 3.0
+ 500 AA	3.12 \pm 0.28**	2.43 \pm 0.55	59.0 \pm 2.0*	34.0 \pm 2.0
+ 1000 AA	5.88 \pm 0.13**	3.07 \pm 1.04	47.0 \pm 2.0	27.0 \pm 2.0
+ 2000 AA	1.85 \pm 0.15**	1.47 \pm 0.25*	45.0 \pm 2.0	21.0 \pm 3.0*

Different from the control group: * $p < 0.05$, ** $p < 0.001$

Discussion

Ascorbic acid is not an essential nutrient for poultry species because they possess the enzyme gulonolactone oxidase that is part of the biosynthetic pathway. However, endogenous synthesis of ascorbic acid may not be adequate to meet the full needs of poultry at all times. Ascorbic acid may be an essential nutrient in poultry when birds are subject to stress conditions because requirements for ascorbic acid are increasing (Whitehead et al., 2003).

The data of our experiment stated that the high loading of ascorbic acid (1000 and 2000 mg/kg) caused a significant increase of MDA and a fall of GSH-Px activity and GSH level in chicken liver. These indices change shows the oxidative stress devel-

opment in tissue. The protein catabolism parameters were enhanced under high level of ascorbic acid intake. However, in case the supplementation of low doses of dietary ascorbic acid was used, a reduction of creatinine and uric acid level in blood serum has been observed. The increasing of ascorbic acid content in diet promoted the accumulation of heavy metal Cd in chicken liver. It can be connected with a rising of intestinal epithelium permeability. The increase of phagocytic activity of blood neutrophils and number of T and B(C3) immunocyte in according immunocompetent organs was observed in the chicken groups that received ascorbic acid addition till 500 mg/kg. It indicated the immunostimulating effect of these ascorbic acid supplements. Further dietary ascorbic acid increase has changed the direction of immune responses in chickens. The indices of innate immunity in blood serum show the immunosuppressive effect of ascorbic acid doses over 1000 mg/kg.

Summary

Ascorbic acid is marketed as a dietary supplement because of its antioxidant properties. However, we report here that ascorbic acid administered to diet in different doses can exhibit a prooxidant, as well as an antioxidant effect in chicks. The high loading of ascorbic acid (1000 and 2000 mg/kg) causes the development of oxidative stress in chicken tissues: increase of MDA and a fall of GSH-Px activity and GSH level. Simultaneously the increase of intestinal epithelium permeability in these chicks is stated. It resulted in enhanced cadmium accumulation in liver. The immunosuppressive effect of ascorbic acid doses over 1000 mg/kg is observed. However, the enrichment of diets by ascorbic acid (100 and 500 mg/kg) stimulates of T- and B(C3)- cell- mediated immune response in chicks.

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Vitamins A, E and antioxidants enzymes in rat liver and heart under different light and geroprotectors influence

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Introduction

The longevity is significantly dependent on reactive oxygen substances the level of which is regulated by the antioxidant system. The antioxidant system of organism include fat-soluble vitamins, such as vitamins E and A, and antioxidant enzymes. Of the many current theories of aging and longevity, the free radical theory (Harman, 1956) has drawn considerable attention. Light conditions influence the lipid peroxidation intensity, which is especially actual for the northern-European population. The changes in circadian biorhythms provoked different physiological disturbances. The circadian rhythm of melatonin in the blood of mammals was known to be functionally linked to the adjustment of 24-hour cycles and to circannual regulation. Melatonin's actions include modulation immune function, tumor growth inhibition and influences on retinal physiology. Light conditions considerably change melatonin synthesis intensity which possesses antioxidant effect and protect organism from accelerated ageing (Reiter et al., 2000).

The aim of this investigation was to study different light condition and geroprotectors (melatonin and epitalon) influence on various antioxidant system components - vitamins E and A level, as well as key antioxidant enzymes activity – superoxide dismutase (SOD) and catalase, in rat liver and heart.

Materials and Methods

Four groups of rats from one to six-month old were kept at 12-hour day and night cycles (Group 1), natural illumination (Group 2), complete darkness (Group 3) and in constant illumination (Group 4). The 4-months old animals from first, second and third groups were divided into three subgroups: first - received melatonin with drinking water in a dose 10 mg/L, second – 0,1 µg of epitalon with hypodermic injection, third

- received placebo. Epitalon is the synthetic analog of epiphysial peptides hormone epitalamin (Anisimov et al., 2002).

The vitamins A and E (α -tocopherol) concentration in the organs was determined by high performance liquid chromatography method. In heart only tocopherol concentration was determined, as the retinol content was not found.

The SOD activity was assessed according to the modified adrenochrome method (Fridovich, 1975), while that of catalase, spectrophotometrically, by the amount of decomposed hydrogen peroxide (Bears, Sizes, 1952). The enzymes specific activity was calculated per 1 g of wet tissue and 1 mg of protein. The protein content was determined by Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard.

The obtained data were processed by standard methods of variation statistics; the comparison was made using non-parametric criteria.

Result and Discussion

The researches had shown that the vitamins A and E concentration and enzymes activity in the investigated organs greater depends on light conditions than on substances.

The vitamin E level in liver in the group under constant illumination (Group 4) was significantly higher compared with the other groups (Fig. 1 A). Probably, the increase in tocopherol concentration under constant illumination is the mechanism of mammals adaptation to unusual light conditions. Melatonin and epitalon reduced vitamins requirement.

The vitamin A content in the Group 1 at 12-hour day and night cycles was significantly higher than in the other groups (Fig. 1 B). In animals kept in complete darkness (Group 3) the vitamin A concentration in liver was the lowest. Low retinol content in liver can be connected with the fact that the most vitamin percentage is utilized for its biological functions, for example, photoreception. Preparations increase vitamin A concentration in Group 1 and decrease its contents in Group 4. Probably, in this case we observed mutual tissue-specific interaction in vitamin metabolism.

The vitamins level in liver was more sensitive to experimental procedure compared with the enzymes activity. There was no influence of preparations on SOD activity under all light conditions. The maximal SOD activity in the liver was observed in the animals kept in complete darkness.

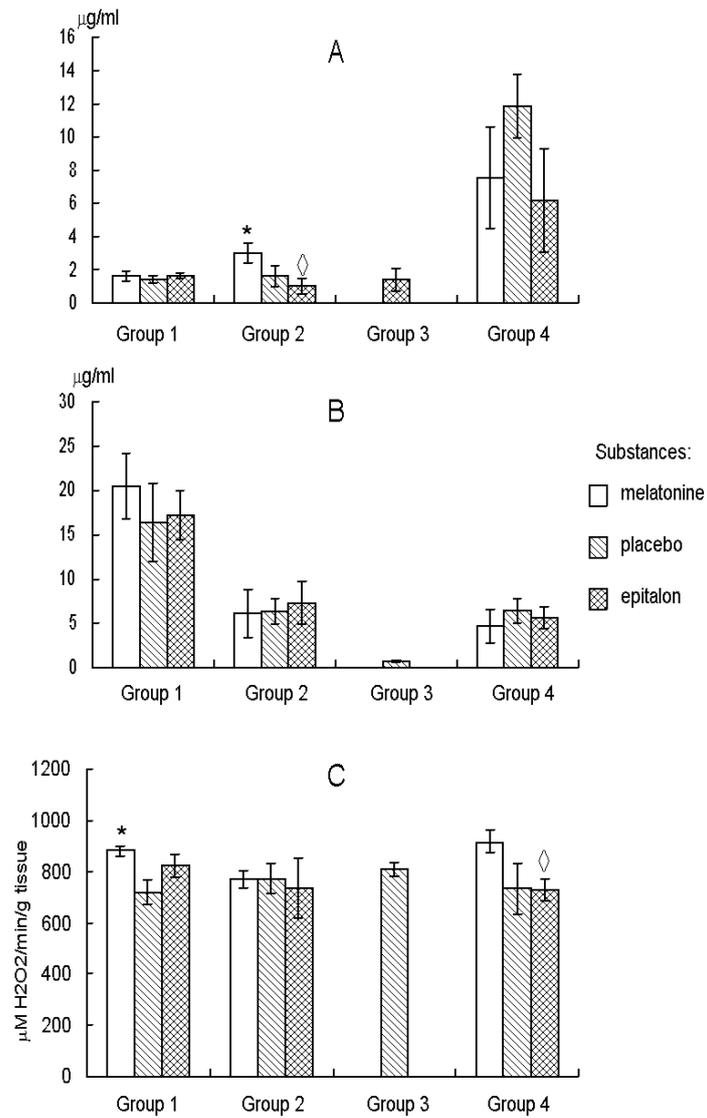


Fig. 1. Vitamin E (A) and A (B) concentration and catalase activity (C) in rat liver under different light conditions. Data presented as mean \pm SD. * - differences is significant in comparison with placebo, \diamond - differences between melatonin and epitalon groups is significant (Mann – Whitney test).

According to multifactor analysis, the catalase activity depended on animal sex as well as on using preparations (Fig. 1 C). Melatonin significantly increased catalase activity, while epitalon did not change it under constant illumination. Similar rising in enzyme activity was found in melatonin group kept in 12-hour day and night cycles. The light conditions did not influence the concentration of vitamin E in heart. Under natural illumination in autumn short light day, epitalon as well as melatonin administration led to the increase α -tocopherol concentration in heart. The rats kept in constant darkness had the lowest vitamin E concentration in heart. These changes can be connected with strong antioxidant effect of endogenous melatonin (Reiter, 2000) or with changes in metabolism intensity under these conditions.

On the other hand, the maximal SOD and catalase activity in heart was observed in the animals kept in constant darkness. At regular 12-hour day and night cycles, significant differences epitalon and melatonin influences on the protein contents in heart in comparison with placebo, have been found, i.e. melatonin increased protein level while epitalon decreased.

The maximum number of significant changes in vitamins concentration and antioxidant enzymes activity under the effect of melatonin and epitalon was found at constant illumination.

Thus, the present research has demonstrated high stability of non-enzyme (vitamins E and A) and enzyme (SOD and catalase) antioxidant system components, to changing light conditions and introducing geroprotectors: melatonin and epitalon.

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Concentrations of 25-hydroxy vitamin D in plasma of dairy cows at different exposure to solar radiation

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Introduction

The exposure to UV-B radiation is a determining factor for the activation rate of vitamin D in the skin (Flachowsky et al., 1993). Because the relevant UV-B radiation increases according to Blumthaler et al. (1992) by 19% per 1000 m of altitude, it may be assumed that cows grazing at high alpine pastures activate more vitamin D in the skin. This would influence the Ca metabolism of the animals, but it could further lead to increased vitamin-D concentrations in the milk. This would give alpine milk a nutritional advantage over milk produced in the lowlands. In order to test the first step of this hypothesis, the concentration of 25-hydroxy-vitamin D in the plasma of cows, being differently exposed to solar radiation, was measured.

Materials and Methods

Period I: At the ETH research station 'Chamau' (400 m a.s.l.) two groups of six lactating Brown Swiss cows each were kept on pasture during calendar weeks 14-19 (Group P) or tethered in a barn (group B). All cows were in their second lactation. Cows were fed exclusively fresh grass and a mineral supplement free of vitamin D and E additions (Ca:P = 2:1; special mixture, Nährkosan, Büron, Switzerland). Group B cows were allowed to move in a paddock every day in the hour before sunrise.

Period II: In calendar weeks 20-23, groups P and B were kept together in a barn and fed freshly cut grass. Three cows of each group were switched to the respective other group. Additionally, six cows, fed a silage-based mixed diet plus concentrates and kept in the same barn, were included in the investigations during this time (group C). None of the cows received extra vitamin D or E. During these weeks, group P was brought out to a shadowed open air paddock (south exposed) between 6.00 and 7.00 a.m., and group B was kept in the same paddock between 11.30 a.m. and 12.30 p.m.

Period III: In calendar week 24, groups P and B were transferred to an alpine research station („Alp Weissenstein“) at 2000 m a.s.l. and were kept and fed similar to period I. In calendar weeks 16, 19, 27 and 30, from each cow blood was sampled twice a week at 1.00 p.m. after fasting the cows for two hours. In period II in calendar weeks 20, 21 and 23, blood was taken once a week following the same principle. The concentration of 25-OH-vitamin D in plasma was determined with a radioimmunoassay (Nichols Institute, USA).

Results

In the beginning of the experiment, from calendar week 16 to 19, the plasma concentrations of 25-OH-vitamin D remained constantly around 28.7 ng/ml (standard error [se] 1.3), a level, which was also found in the baseline evaluation in calendar week 12 (Fig. 1). In calendar week 19, the level increased to 32.1 (se 1.0) in group P, whereas it decreased in group B to 25.3 (SF 1.4) ng/ml. In calendar week 19, this caused a significant difference between grazing or feeding freshly cut grass ($p < 0.05$), but this difference was not further observed in the later stages of the experiment. Group C cows had an average (baseline) concentration of 25.0 ng/ml in calendar week 19. On average of all groups, the values increased significantly from week 19 to week 20 (from 27.3 to 38.4 ng/ml, $p < 0.01$) and from week 20 to week 21 (from 38.4 to 46.9 ng/ml, $p < 0.01$). During the same time, solar radiation, measured at noon, increased from 400 Watt/m² in weeks 16 and 19 to 850 Watt/m² in week 20. Although the radiation spectrum between 300 and 3000 nm was not analyzed in detail, we assume, that this also indicates a considerable increase of the UV-B radiation (Stick, 1998). From week 21 to week 23 the values varied differently in each group, and increased particularly in group C from 39.9 (se 1.0) to 72.7 ng/ml (se 8.6), regardless of the daytime, when the cows of this group were moved to the open air paddock. The values of groups P and B reached a plateau of 47.6 ng/ml (se 5.1) at that time, which was also not systematically affected by the daytime staying in the open. At the beginning of period III, the 25-OH-vitamin D concentrations of the plasma were again relatively lower (average of P and B 37.4 ng/ml; se 3.4) but further on they increased continuously and reached 48.7 ng/ml (SF 11.1) in week 30. However, the large standard error shows that the between-animal variation was much more pronounced than in periods I and II.

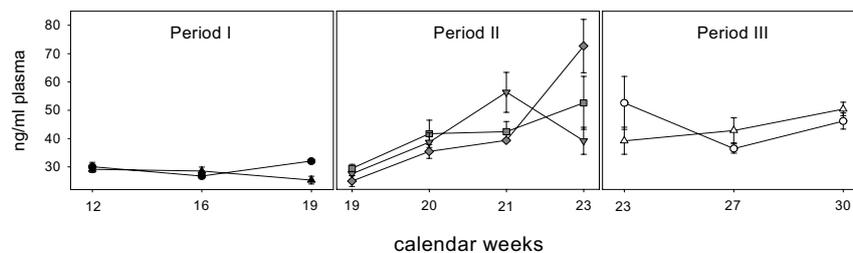


Figure 1. Concentrations of 25-OH-vitamin D in plasma of cows in the three experimental periods. Periods I and III: ●, pasture group; ▲, indoor group. Period II: ■, grass-fed group, let out in the morning; ▼, grass-fed group, let out at noon; ◆, silage-concentrate group, let out at noon.

Discussion

Other than expected from the increase in UV-B radiation with increasing altitude (Blumthaler, et al., 1992), the alpine sojourn did not cause higher concentrations, of 25-OH-vitamin D in plasma of cows than those found in the lowlands. In contrast to the results of Flachowsky et al. (1993), grazing in the lowlands, in comparison to feeding in barn, had a positive effect on the 25-OH-vitamin D concentrations only in one week. We assume that the absence of a clearer effect was caused by the one hour movement of group B cows under the sky in each morning. According to Stick (2001), the diffuse UV-B radiation (which also reaches the barns through the windows) is even stronger than the direct radiation. We also did not find an effect of the daytime when cows put outside. This result is surprising, because the radiation of the relevant wavelengths (about 295 nm) should be much more intensive at noon than in the early morning (Stick, 1998).

Regardless of the experimental factors, the general evolution of the plasma 25-OH-vitamin D concentrations during the time of the experiment was similar to that observed by Flachowsky et al. (1993) during summer on pasture in northern Germany. In the investigations of Flachowsky et al. (1993) and Hidiroglou et al. (1979), the 25-OH-vitamin D concentration in plasma of dairy cows did not increase beyond 47 ng/ml, this even not after oral or intravenous application of 1 million I.U. vitamin D₃. However, Flachowsky et al. (1993) found considerable amounts of vitamin D₃ after the application in plasma and in milk. This could serve as a possible explanation for the lack of differences in the current study. If, even with increased

plasma concentrations of non-hydroxylated vitamin D, the hydroxylation in the liver would be limited to a certain extent, one could expect an increased excretion of the non-hydroxylated form with milk, as was observed by Flachowsky et al. (1993). However, the extent of secretion of non-hydroxylated vitamin D with milk in the current study could not be determined since levels were not detectable and thus this hypothesis still has to be verified.

Summary

Eighteen lactating dairy cows were exposed to solar radiation to different extents. Tested treatments were grazing on pasture vs. feeding indoors, different daytimes of exposure to daylight (one hour during sunrise vs. one hour at noon, while remaining the rest of the day in barn), and lowland grazing (at 400 m a.s.l.) vs. high alpine grazing (at 2000 m a.s.l.). No direct effects of these treatments on the 25-OH-vitamin D concentration in the blood plasma of the cows were found. A general increase of the concentrations with the onset of the warm summer weather was found in all groups. It is concluded that the concentration of the hydroxylated form of vitamin D does not respond to the treatments investigated. However, this does not allow to assume the same for the non-hydroxylated form of vitamin D, because the lack of effects also could be caused by a limited hydroxylation capacity of the liver.

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Studies on the biotin flow at the duodenum of dairy cows fed differently composed rations

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Introduction

It is well established that rumen microbes are able to synthesize B-vitamins, but knowledge of the effects of feed composition on net vitamins output is scarce. One of the B-vitamins of interest for ruminants is biotin. However, literature data on biotin flow at the duodenum vary considerably (NRC, 2001; Santschi et al., 2005). There is evidence from *in vivo* studies in steers that the portion and the type of grain in the diet influence the biotin metabolism in the rumen, resulting in changes in the duodenal flow of the vitamin (Miller et al., 1986). The objective of the study reported herein was to measure the biotin flow at the proximal duodenum of dairy cows either receiving pure hay- or mixed roughage/concentrate-rations with corn silage or grass silage, the latter being supplemented with high amounts of wheat- or corn-based concentrate, and to prove if there will be a correlation to the degree of microbial activity in the rumen.

Materials and Methods

Two dry (Diet 1) and four lactating (Diets 2 to 4) dairy cows, each fitted with cannulae in the rumen and in the proximal duodenum were used in four trials. On dry matter basis Diet 1 consisted of 8.9 kg grass hay, Diet 2 of 8.9 kg corn silage and 2.0 kg protein-mixture and Diets 3 and 4 of 7.4 and 7.3 kg grass silage and 10.0 kg concentrate mixture containing 87% wheat or corn grain respectively, 11% soybean meal and 2% oil. Mineral-vitamin premix in the concentrates was free of biotin. Diets were given restrictively to avoid feed refusals and administered in two equal sized meals. Concentrate of Diets 3 and 4 was given in four meals.

Each four-week experimental period (one per diet) consisted of two weeks for adaptation to the diet, one week during which ruminal fluid for three days (180 min. after the start of morning feeding) was collected for the determination of pH, ammonia and

short chain fatty acids (SCFA) and one week for duodenal sample collection according to Rohr et al (1979). To estimate digesta flow at the duodenum, Cr₂O₃ mixed with wheat flour was used (Rohr et al., 1984) and microbial N in the duodenal chyme was determined by near infrared spectroscopy (NIRS) as described by Lebzien and Paul (1997). Biotin in feedstuffs and duodenal digesta was measured applying a microbiological method (Frigg and Brubacher, 1976).

Results

The rumen fermentation data are shown in Table 1.

Table 1. umen fermentation parameters 180 min after the start of morning feeding (means and standard deviations)

Diet (n)	Grass hay	Corn silage + concen- trate	Grass silage + wheat based concentrate	Grass silage + corn based concentrate
	1 (6)	2 (12)	3 (9)	4 (9)
pH	6.74 ^a ± 0.18	6.20 ^b ± 0.15	5.47 ^d ± 0.26	5.82 ^c ± 0.39
NH ₃ -N (mg/100ml)	10.8 ^b ± 0.4	12.8 ^b ± 3.3	6.3 ^c ± 3.0	17.8 ^a ± 6.2
SCFA (mmol/l)	80.9 ^d ± 15.6	96.6 ^c ± 8.3	143.8 ^a ± 18.4	134.7 ^b ± 16.2
molar proportion (%)				
acetate	71.7 ^a ± 1.6	68.0 ^a ± 4.6	56.9 ^b ± 2.7	59.0 ^b ± 3.2
propionate	16.4 ^c ± 0.9	16.3 ^c ± 2.0	28.1 ^a ± 5.3	22.7 ^b ± 3.3
butyrate	8.1 ^c ± 1.1	11.1 ^b ± 2.3	15.4 ^a ± 3.1	14.3 ^a ± 1.1
acetate : propionate	4.4 : 1 ^a	4.2 : 1 ^a	2.1 : 1 ^c	2.7 : 1 ^b

a > b > c > d in the same line: p < 0.05

The pH value decreased and short chain fatty acid concentration increased with an increasing concentrate portion in the diet which was more pronounced in combination with the wheat than with the corn grain based concentrate. Ammonia concentration was significantly lower with Diet 3 and significantly higher with Diet 4 as compared to Diets 1 and 2. Acetate : propionate ranged from 2.1 : 1 (Diet 3) to 4.4 : 1 (Diet 1). Daily means for the intake of organic matter (OM), crude protein (CP) and biotin, as well as the average amounts of OM fermented in the rumen (FOM) and of microbial nitrogen (MN) and biotin at the duodenum are summarized in Table 2.

The percentage of FOM was significantly higher when feeding the concentrate rich diet with wheat grain (Diet 3) as compared to Diet 1 or 4. Efficiency of microbial protein synthesis (g MN/kg FOM) increased with increasing DM intake.

Table 2. Intake of organic matter (OM), crude protein (CP) and biotin, OM fermented in the rumen (FOM)¹ as well as flow of microbial nitrogen (MN) and biotin at the proximal duodenum

Diet (n)	Grass hay	Corn silage + concen- trate	Grass silage + wheat based concentrate	Grass silage + corn based concentrate
	1 (2)	2 (4)	3 (3)	4 (3)
Intake				
OM (kg/d)	8.33	10.27	16.12	16.29
CP (g/d)	1188	1412	2849	2646
Biotin (mg/d)	2.55	2.35	4.49	4.20
(mg/kg DM)	0.29	0.22	0.25	0.24
FOM ¹ (kg/d)	5.94 ± 0.09	7.83 ± 0.39	13.13 ± 0.32	11.59 ± 0.65
(% of OM intake)	71.3 ^b ± 1.06	76.2 ^{ab} ± 3.76	81.5 ^a ± 2.02	71.1 ^b ± 3.94
Flow at the duodenum				
MN (g/d)	120.0 ± 9.5	174.6 ± 32.3	395.1 ± 17.3	356.1 ± 37.2
(g/kg FOM)	20.2 ^b ± 2.2	22.3 ^b ± 3.6	30.9 ^a ± 1.9	30.5 ^a ± 1.6
Biotin (mg/d)	1.84 ± 0.62	4.37 ± 0.41	5.89 ± 1.35	6.16 ± 0.93
(mg/kg FOM)	0.31 ^b ± 0.10	0.56 ^a ± 0.05	0.45 ^{ab} ± 0.09	0.53 ^a ± 0.05
(µg/g MN)	15.4 ^{ab} ± 6.7	25.0 ^a ± 3.6	14.9 ^b ± 2.8	17.3 ^b ± 1.1
Biotin flow – biotin in- take (mg/d)	-0.71	2.02	1.40	1.96

a > b: p < 0.05

¹FOM = OM-intake – (OM flow at the duodenum – microbial OM) where microbial OM = microbial N x 11.8 (Schafft 1983)

Biotin intake per kg DM was roughly the same for the four diets (0.22 – 0.29 mg/kg DM). In consequence the biotin intake depended primarily on the level of feed intake. Biotin flow at the duodenum was lowest with Diet 1. Consequently, the biotin balance calculated as difference between flow at the duodenum and intake was negative in the hay fed cows (Diet 1) and positive in association with the other three diets.

Discussion

The amounts of biotin determined in the present investigations leaving the rumen may give some hints as to ruminal biotin degradation/utilisation and ruminal microbial net synthesis in cows supplied with different amounts of dietary biotin and differently composed feed rations entailing large variations in ruminal fermentation conditions. In agreement with other studies (Miller et al., 1986; Zinn et al., 1987), the positive biotin balances observed in our study clearly confirm biotin synthesis by rumen microbes. In contrast Santschi et al. (2005) could not observe a net biotin synthesis in the rumen. Biotin flow at the duodenum was not significantly (p > 0.05) related to the

amounts of consumed biotin ($r = 0.63$) but to the amount of organic matter fermented in the rumen ($r = 0.85$) and to the amount of synthesised microbial protein ($r = 0.84$). More duodenal biotin per kg FOM was measured in cows supplied with the mixed diets. These results agree with earlier observations in fistulated steers (Miller et al., 1986), and may be associated with the shift of propionate production from biotin-dependent randomising to the biotin-independent acrylate pathway when easily fermentable carbohydrates are supplied with the concentrate. Overall mean daily biotin flow was 0.48 ± 0.11 mg/kg FOM. Taking a mean daily biotin requirement of 6 mg, given by NRC (2001) for a dairy cow (650 kg LW) with 35 kg fat corrected milk, into account it can be calculated, that about 12.5 kg OM have to be fermented.

Summary

Dairy cows fitted with permanent cannulas in the dorsal rumen and in the proximal duodenum were fed differently composed diets, and the biotin flow at the proximal duodenum was measured. The diets (on DM basis) consisted of 8.9 kg grass hay (Diet 1), 8.9 kg corn silage plus 2.0 kg concentrate (Diet 2), or 7.3 and 7.4 kg grass silage plus 10.0 kg concentrate (Diets 3 and 4). The concentrate in Diets 3 and 4 contained 87% wheat and corn grain, respectively. The duodenal flow of biotin was not significantly related to biotin intake, but to the amount of fermented organic matter (FOM) and the amount of microbial protein ($r = 0.85$ and $r = 0.84$), irrespective of the composition of the diet fed. Mean daily biotin flow was 0.48 ± 0.11 mg/kg FOM without any systematic effect of diet composition.

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Effects of chronic ethanol treatment on levels of vitamin B1, B2, and B6 in rat brain

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Introduction

Vitamin B1 (thiamine) and vitamin B6 (pyridoxine) act in form of their coenzymes (thiamine diphosphate [TDP] and pyridoxal 5'-phosphate [PLP], respectively) in the carbohydrate and amino acid metabolism. Vitamin B2 (riboflavin) catalyses numerous oxidation-reduction reactions. (Figure 1) The major function of riboflavin is to serve as the precursor of the coenzymes FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) and of covalently bound flavins. Because FAD is part of the respiratory chain, vitamin B2 is central to energy production. The aim of the present study was to investigate the long-term ethanol related effects on vitamin B1, B2, and B6 status in brain tissue, to get new facts for a potential therapeutically treatment.

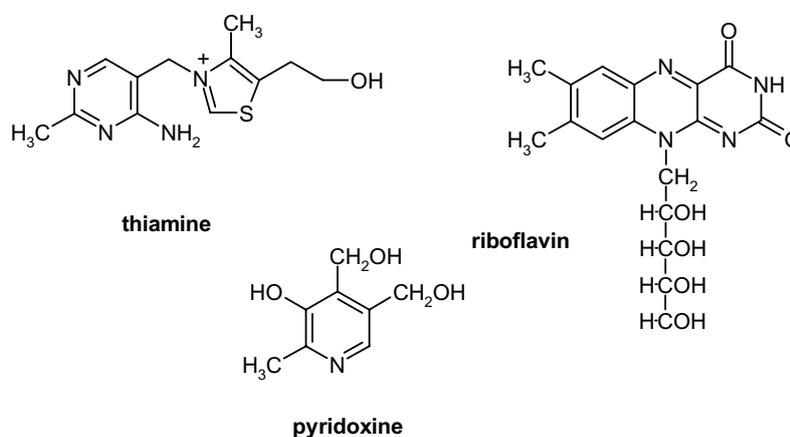


Figure 1. Chemical structures of the investigated B-vitamins.

Material and Methods

Male Wistar rats (n = 14) were separated into a control group (n = 7) and an alcohol group (n = 7) at random. The animals received a semi synthetic diet (11.9 mg Thiamine Hydrochloride, 18.2 mg Pyridoxine, and 7.5 mg Riboflavin per kg diet), which was sufficient in all essential nutrients. Alcohol (20% ethanol-solution) was given instead of tap water. The ethanol calories were substituted by sucrose and the treatment groups were fed isocalorically for 12 months.

The B-vitamins (as a sum of all detectable vitamers, respectively) were analysed in brain tissue by RP-HPLC: Vitamin B1 (thiamine, thiamine monophosphate (TMP), and TDP) according to Netzel, 1997; vitamin B2 (FAD, FMN, and riboflavin) according to Zemleni et al., 1992 and Yagi & Sato, 1981; vitamin B6 (pyridoxine, pyridoxal, pyridoxamine, PLP, and 4-pyridoxic acid) according to Kimura et al., 1996.

Results

Chronic ethanol intake caused significantly ($p < 0.05$) reduced levels of vitamin B6 (-19 %), PLP (-26 %), FAD and FMN (-18 %), as well as of TDP (-21 %). Vitamin B1 and B2 (as a sum of all detectable vitamers, respectively) were slightly reduced ($p > 0.05$) of about 4 and 12 %, respectively (Table 1 and Figures 2-4).

Table 1. Concentrations of vitamin B1, B2, and B6 in brain tissue of the rats treated under the described conditions.

vitamins	alcohol-free control group	alcohol group
vitamin B1 (ng/g)	2099.3 ± 105.3	2011.6 ± 108.5
TDP (ng/g)	1846.1 ± 255.4*	1473.7 ± 162.9
vitamin B2 (nmol/g)	28.3 ± 5.4	25.0 ± 5.1
FAD/FMN (nmol/g) ¹	25.8 ± 2.1*	21.3 ± 2.5
vitamin B6 (nmol/g)	17.2 ± 1.8*	13.9 ± 1.3
PLP (nmol/g)	13.8 ± 1.6*	10.2 ± 1.9

¹FAD was converted to FMN; *statistically significant, $p < 0.05$ (alcohol-free control group vs. alcohol group).

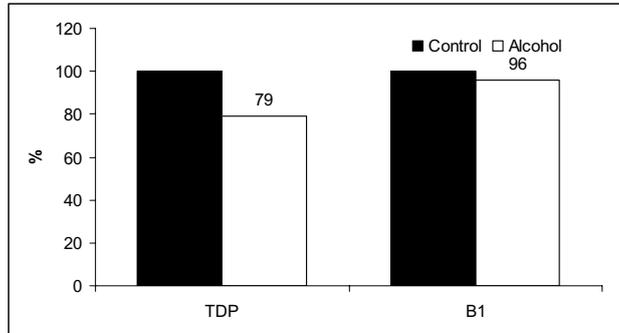


Figure 2. Changes (%) in TDP and vitamin B1 compared to the ethanol-free control (brain tissue).

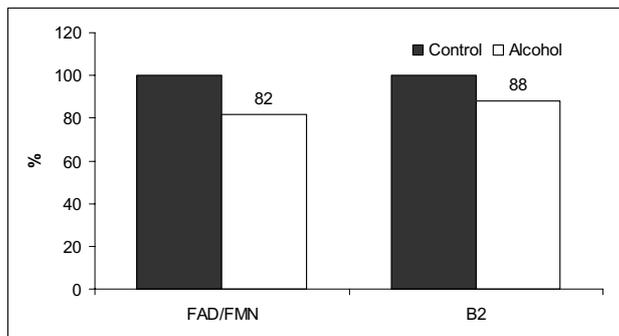


Figure 3. Changes (%) in FAD/FMN and vitamin B2 compared to the ethanol-free control (brain tissue).

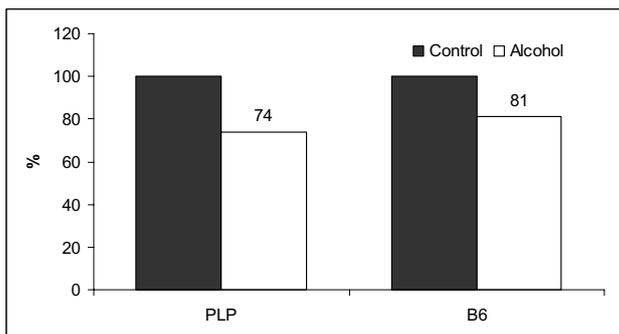


Figure 4. Changes (%) in PLP and vitamin B6 compared to the ethanol-free control (brain tissue).

The results suggest that ethanol markedly impairs the conversion of Thiamine, Riboflavin, and Pyridoxine to their physiological active coenzymes, which may contribute to the depression of brain metabolism observed in alcoholism.

Summary

The aim of the present study was to investigate the long-term ethanol related effects on vitamin B1, B2, and B6 status in brain tissue of male wistar rats, to get new facts for a potential therapeutically treatment. The B-vitamins were analysed by HPLC. Chronic ethanol intake caused significantly ($p < 0.05$) reduced levels of vitamin B6 (as a sum of all detectable vitamers), PLP (coenzyme of B6), FAD/FMN (coenzymes of B2), as well as of TDP (coenzyme of B1). Vitamin B1 and B2 (as a sum of all detectable vitamers, respectively) were slightly reduced ($p > 0.05$). The results suggest that ethanol markedly impairs the conversion of thiamine, riboflavin, and pyridoxine to their physiological active coenzymes, which may contribute to the depression of brain metabolism observed in alcoholism.

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Efficacy of benfotiamine vs. thiamine hydrochloride on thiamine status in ethanol treated rats

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Introduction

Thiamine deficiency is often related to chronic alcohol consumption. An insufficient intake of the vitamin, decreased formation of the biological active coenzyme, reduced hepatic storage and inhibition of intestinal thiamine transport by ethanol are the circumstances which may be responsible for this association (Laforenza et al., 1990; Bitsch et al., 1982; Hoyumpa, 1980). Benfotiamine, one of the most important synthetic lipophilic thiamine derivatives (Figure 1), is used for the treatment of atypical neuralgias, polyneuropathies and encephalopathias caused by chronic alcohol abuse, diabetes mellitus or gastrointestinal dysfunctions. The purpose of the present investigation was to study the efficacy of benfotiamine, compared to thiamine hydrochloride (water-soluble salt), on thiamine status in ethanol treated rats.

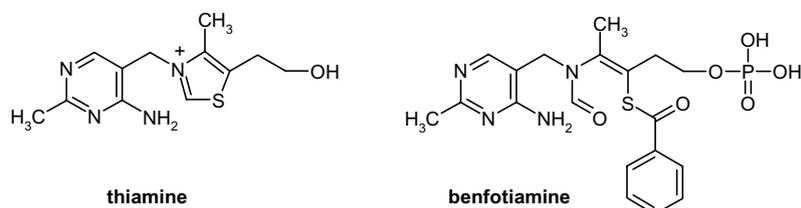


Figure 1. Chemical structures of thiamine and benfotiamine

Material and Methods

The study was performed with 21 male Wistar rats, which were separated into 3 treatment groups (7 animals each) at random. Group C (control): thiamine hydrochloride (11.9 mg/kg diet) and tap water; group TE (thiamine/ethanol): thiamine hydrochloride (11.9 mg/kg diet) and ethanol (20%-solution) per oral ad libitum; group BE (benfotiamine/ethanol): benfotiamine (16.45 mg/kg diet; equimolar to thiamine hydrochloride in the treatment groups TE and C) and ethanol (20%-solution) per oral ad libitum. The ethanol calories were substituted by sucrose and the groups were fed

isocalorically for 3 months. Thiamine, thiamine monophosphate (TMP), and thiamine diphosphate (TDP; biological active coenzyme) were determined in blood, brain, heart, liver and kidney by HPLC (Netzel, 1997).

Results

The ethanol treatment caused significantly ($p < 0.05$) reduced levels of TDP in blood (-22 %), liver (-24 %) and kidney (-17 %), and of total thiamine (sum of TDP, TMP, and thiamine) in blood (-14 %) and liver (-19 %), respectively. An improved TDP and total thiamine status could be observed in the benfotiamine fed animals (compared with the ethanol-free control as well as with the thiamine hydrochloride/ethanol group) (Table 1 / Figure 2 and 3).

Table 1. Concentrations of TDP, TMP, thiamine, and total thiamine in blood and tissue of the rats treated under the described conditions.

matrix	gr.	TDP (ng/ml ; ng/g)	TMP (ng/ml ; ng/g)	thiamine (ng/ml ; ng/g)	tot. thiamine (ng/ml ; ng/g)
blood	C	125.6 ± 10.6	51.2 ± 8.0	54.8 ± 3.0	172.9 ± 7.0
	TE	97.7 ± 14.3	47.8 ± 10.0	51.5 ± 8.0	149.5 ± 6.6
	BE	173.5 ± 12.2	38.3 ± 9.3	54.2 ± 3.8	192.2 ± 6.3
sig. *p < 0.05	C : TE*; C : BE*; TE : BE*		C : BE*	-	C : TE*; C : BE*; TE : BE*
brain	C	1839.5 ± 71.1	607.1 ± 42.6	97.9 ± 8.3	1714.8 ± 27.6
	TE	1790.6 ± 92.4	632.7 ± 42.6	96.7 ± 10.3	1702.7 ± 22.6
	BE	2006.7 ± 94.4	610.8 ± 52.6	96.3 ± 11.1	1820.4 ± 38.9
sig. *p < 0.05	C : BE*; TE : BE*		-	-	C : BE*; TE : BE*
heart	C	4812.1 ± 224.1	1317.7 ± 158.8	121.1 ± 23.3	4143.2 ± 78.1
	TE	4472.8 ± 257.6	1466.1 ± 202.7	87.1 ± 15.5	4010.5 ± 73.1
	BE	5145.4 ± 358.0	1405.0 ± 226.7	113.5 ± 17.6	4410.2 ± 186.9
sig. *p < 0.05	TE : BE*		-	C : TE*; TE : BE*	C : BE*; TE : BE*
liver	C	4754.0 ± 211.1	1342.3 ± 154.1	448.5 ± 95.6	4452.4 ± 174.0
	TE	3623.0 ± 385.5	1311.3 ± 156.8	345.4 ± 86.1	3618.6 ± 230.8
	BE	5341.9 ± 187.3	1524.6 ± 241.8	506.9 ± 76.0	5018.5 ± 193.3
sig. *p < 0.05	C : TE*; C : BE*; TE : BE*		-	TE : BE*	C : TE*; C : BE*; TE : BE*
kidney	C	2537.7 ± 331.5	490.1 ± 63.3	192.0 ± 40.2	2155.2 ± 162.6
	TE	2113.3 ± 190.3	508.5 ± 42.4	210.5 ± 43.6	1922.5 ± 110.6
	BE	2610.5 ± 308.2	488.6 ± 46.5	235.4 ± 26.4	2248.0 ± 207.1
sig. *p < 0.05	C : TE*; TE : BE*		-	-	TE : BE*

Treatment groups: C = ethanol-free control, TE = thiamine hydrochloride and ethanol, BE = benfotiamine and ethanol

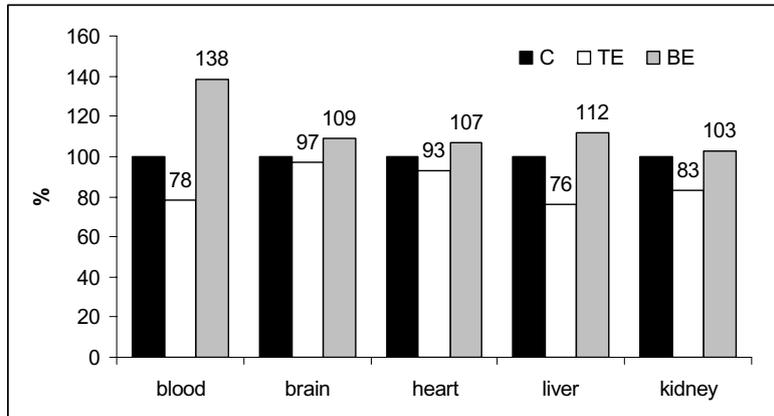


Figure 2. Changes (%) in TDP compared to the ethanol-free control

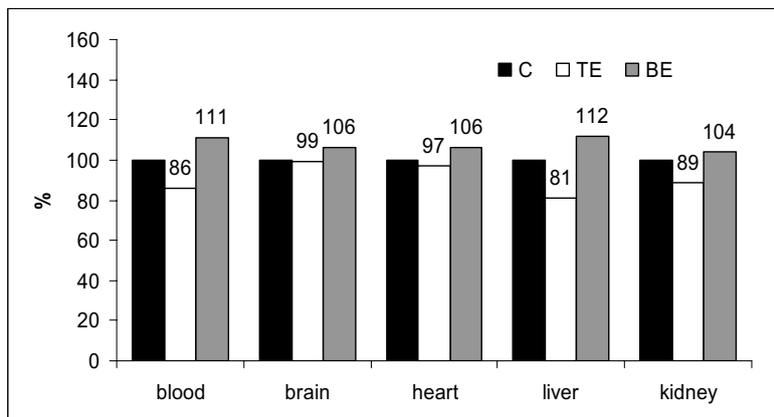


Figure 3. Changes (%) in total thiamine compared to the ethanol-free control

Treatment with benfotiamine could be an effective strategy in the prevention of alcohol induced thiamine/ TDP deficiency.

Summary

The purpose of the present investigation was to study the efficacy of benfotiamine (lipid-soluble thiamine derivative), compared to thiamine hydrochloride (water-soluble salt), on thiamine status in ethanol treated rats. The ethanol treatment caused significantly reduced levels of thiamine diphosphate (TDP) in blood, liver and kidney, and of total thiamine (sum of TDP, TMP, and thiamine) in blood and liver, respectively. An improved TDP and total thiamine status could be observed in the benfotiamine/ethanol fed animals (compared with the ethanol-free control as well as with the thiamine hydrochloride/ethanol group). Treatment with benfotiamine could be an effective strategy in the prevention of alcohol induced thiamine/ TDP deficiency.

Acknowledgement

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Pflanzliches Legehennenfutter ohne und mit Vitamin B₁₂-Supplementation

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Einführung

Die Mischfuttermittel für Geflügel werden alle mit Vitamin B₁₂ supplementiert. In Tabellen mit Richtwerten für Vitamingehalte erfolgt keine Angabe zur B₁₂-Konzentration in Futtermitteln pflanzlicher Herkunft. In rein pflanzlichen Rationen wird aber auch Vitamin B₁₂ analysiert, welches in anhaftenden Mikroben enthalten ist. Unter Vitamin B₁₂ werden alle potenziell biologisch aktiven Cobalmine zusammengefasst, nicht vitaminwirksame werden als Pseudocobalmine bezeichnet. Fische nehmen z. B. B₁₂ mit Algen auf, die an der Oberfläche Bakterienkulturen haben (Kolb und Seehawer, 2003). Algen enthalten 2 - 170 µg B₁₂/100 g Trockensubstanz (Friedrich, 1975). In rein pflanzlichem Legehennenfutter in Mehlform der vorliegenden Versuche wurden 5,3 µg B₁₂ und Analoge/kg analysiert. Strauß (2004) fand in verschiedenen Getreidesorten 11-128 µg B₁₂/kg und eine gute Korrelation zur Mikroorganismen-Keimzahl. In 1 g Trockensubstanz E.coli sind etwa 1 µg B₁₂, in 1 g aerober Bakterien 10 µg B₁₂, in 1 g anaerober Bakterien 800 - 1000 µg B₁₂ und in 1 g mittlerer Bakterienbesatzes von Mischfutter bis 10 µg B₁₂ jeweils inklusive Analoge enthalten. Auch in Faeces sind erhebliche Mengen an B₁₂ nachweisbar, die über Koprophagie zur B₁₂-Versorgung des Tieres beitragen. Die Enteroflora produziert allerdings einen nicht unbedeutenden Anteil an Pseudocobalminen (Bitsch, 2004). Roth-Maier und Paulicks (2002) haben in Broilerversuchen mit nur pflanzlichen Rationen ohne B₁₂-Zusatz keine nachteiligen Folgen auf die zootecnischen Leistungen im Vergleich zur Kontrolle mit B₁₂-Supplementation nachweisen können.

Material und Methoden

In einem 40-wöchigen Versuch mit 5 x 2 LSL-Hennen und einem 22-wöchigen Versuch mit 8 x 2 LB-Hennen jeweils/Gruppe in Käfighaltung im Alter von 24 - 64 bzw. 38-60 Wochen wurde eine pflanzliche Basalration in Mehlform (Tab. 1) ohne und mit 20 µg B₁₂-Zusatz/kg Mischfutter (MF) angeboten. Die Hennen sind am Versuchsanfang und 8-wöchentlich gewogen worden. Die Erfassung der Eizahl erfolgte täglich, die der Eimasse an 3 Tagen/Woche. Im Versuchszeitraum sind kontinuierlich jeweils

von 8 x 10 Eiern/Gruppe äußere und innere Eiquantitätsmerkmale gemessen worden. Außerdem erfolgte eine viermalige Bonitierung des Gefieders der Einzeltiere und eine sechswöchige TS-Bestimmung einer Exkrementensammelprobe/Gruppe. Im lyophilisierten essbaren Ei (6 x 4 Eier) und Leber (4 x 2 Lebern) sowie bei 60° C getrockneten Exkrementen (5 Proben) und Caecuminhalt (3 Proben jeweils/Gruppe) wurde die B₁₂-Konzentration analysiert. Die Bestimmung der B₁₂-Aktivität erfolgte nach der mikrobiologischen Methode mit dem Milchsäurebakterium *Lactobacillus leichmannii* (ATCC 7830) und erfasst auch Vitamin B₁₂-Analoge. Im Mittel wurden in den Basalrationen ohne Zusatz 5,3 µg B₁₂ und in den Versuchsrationen 30,3 µg B₁₂/kg Legehennenfutter analysiert (n = 3).

Tabelle 1: Zusammensetzung und wertbestimmende Inhaltsstoffe der eingesetzten Rationen (g/kg Mischfutter)

Komponenten	Versuch	Versuch	Gehalte	Versuch	Versuch
	A	B		A	B
Weizen	570,4	258,5	Energie (MJ ME)	10,9	11,1
Gerste	-	190	Rohprotein	178	175
Mais	-	150	Rohasche	122	135
Sonnenblumenex-schrot	110	-	Rohfaser	52	38
Sojaextraktionsschrot	176,7	255,9	Rohfett	62	60
Soja-Öl	40	14,1	Stärke	338	352
Bergafat HTL 306	-	20	Zucker	29	37
Lysin-HCl	1,1	-			
Methionin	0,6	0,7			
Ca-Na-Phosphat	4,3	7,6			
Kalkstein	76,9	83,2			
Mineralfutter	20	20			

Ergebnisse und Diskussion

Die unterschiedliche Vitamin B₁₂-Versorgung der Legehennen nahm keinen signifikanten Einfluss auf die zootechnischen Leistungen (Tab. 2). Die Hennen ohne B₁₂-Supplementation erzielten im Mittel der zwei Versuche 0,4 %-Punkte geringere Legeleistung mit 1,4 g höherer Einzeleimasse, im Versuch B signifikant. Somit erreichten die nicht supplementierten Hennen 1,8 % höhere Eimasseproduktion (p > 0,05). Die Futtermittelverwertung betrug in den beiden Gruppen 1,97 vs. 1,93. Auch in der Körpermassezunahme und der Bonitierung des Gefieders bestanden nur unwesentliche

Unterschiede. Die Tierabgänge infolge Mortalität und Selektion lagen in der nicht-supplementierten Gruppe höher. Bei einer selektierten Henne in der Gruppe ohne Zusatz konnten nervöse Störungen, Lähmungserscheinungen und Kachexie diagnostiziert werden. Erst 3 Tage vor der Selektion stellte diese Henne die Legetätigkeit ein.

Tabelle 2: Leistungen von Legehennen mit und ohne Vitamin B₁₂-Zusatz

Parameter	Versuch A				Versuch B			
	+ 20 µg B ₁₂ /kg MF		ohne Zusatz		+ 20 µg B ₁₂ /kg MF		ohne Zusatz	
	3	s %	3	s %	3	s %	3	s %
Futtermittelverzehr, g/Tier und Tag	113,2	2,0	114,7	3,5	123,0	9,0	120,4	2,9
Futtermittelaufwand, kg/kg Eimasse	1,95	0,08	2,03	0,16	2,00	0,20	1,85	0,09
Legeleistung, %	92,8	3,0	91,1	6,2	93,1	6,9	94,0	4,1
Einzeleimasse, g	62,7	1,8	62,1	3,2	66,0	2,2	69,4*	2,7
Zunahme, g	183	78	236	84	58	108	92	123
Tierabgänge, %	0		10	22	6,2	17,7	6,2	17,7
Beurteilung des Gefieders	1,7	0,7	0,7	1,8	1,8	0,7	1,8	0,7

* signifikant zur supplementierten Gruppe, p < 0,05

Tabelle 3: Eiqualität bei Legehennen mit und ohne Vitamin B₁₂-Zusatz

Parameter	Versuch A				Versuch B			
	+ 20 µg B ₁₂ /kg MF		ohne Zusatz		+ 20 µg B ₁₂ /kg MF		ohne Zusatz	
	3	s %	3	s %	3	s %	3	s %
Deformation, µm	57	15	58	12	65	12	60**	12
Bruchfestigkeit, N	46	10	46	7	39	9	43**	10
Schalendichte, mg/cm ²	92,7	6,4	92,4	11,0	87,2	5,4	91,7**	7,4
Schalenfarbe, Reflex., %	-		-		33	6	29**	6
Eiklarhöhe, mm	6,8	1,2	7,0	1,4	5,2	1,6	5,7*	1,6
Haugh-Einheiten, HU	81	9	83	8	65	17	69*	15
Blutflecken, %	0		1,2		14,1		18,4	

* signifikant zur supplementierten Gruppe p < 0,05, ** p < 0,01

In den äußeren und inneren Eiquantitätsmerkmalen wurden im Versuch A keine signifikanten Unterschiede gemessen. Im Versuch B waren die Bruchfestigkeit, die Dichte der Eischalen, die Eiklarhöhe sowie die Haugh-Einheiten in der Gruppe ohne B₁₂-Zusatz signifikant höher und die Eischalen dunkler (Tab. 3).

Der Trockensubstanzgehalt der Exkreme lag in den Gruppen ohne Zusatz höher als in denen mit B₁₂-Supplementation, im Versuch A und im Mittel der beiden Versuche signifikant. Im Eiinhalt, in der Leber und im Caecuminhalt bestanden keine signifikanten Beziehungen zwischen Trockensubstanz und Fütterung (Tab. 4).

Tabelle 4: Trockensubstanzgehalt in verschiedenen Substanzen, in %

Stoff	Versuch A		Versuch B	
	+ 20 µg B ₁₂ /kg MF	ohne Zusatz	+ 20 µg B ₁₂ /kg MF	ohne Zusatz
essbares Ei	24,4	24,4	24,4	23,6
Leber	31,6	31,9	32,0	33,6
Caecuminhalt	26,0	24,9	26,4	25,8
Exkreme	22,8	25,0	24,8	25,2

* signifikant zur supplementierten Gruppe, p < 0,05

Im Eiinhalt stieg die Vitamin B₁₂-Konzentration in der Gruppe mit Vitamin B₁₂-Supplementation des Futters im Mittel der Versuche um 19 % und in der Leber um 66 % signifikant an (Tab. 5). Auch in den Exkrementen war bei den supplementierten Kontrollhennen 49 % mehr B₁₂ enthalten (p > 0,05). Im Caecum erfolgte eine intensive B₁₂-Synthese.

Tabelle 5: Vitamin B₁₂-Konzentration in verschiedenen Substanzen (µg/kg Trockensubstanz)

Stoff	Versuch A			Versuch B				
	n	+ 20 µg B ₁₂ /kg MF	ohne Zusatz	n	+ 20 µg B ₁₂ /kg MF		ohne Zusatz	
		3	3		3	s %	3	s %
essbares Ei	2	178	160	4	188	10	152*	12
Leber	2	2.295	1.000	2	2.260	-	1.750	-
Caecuminhalt	1	24.630	31.100	2	17.270	-	16.920	-
Exkreme	1	1.097	1.208	4	2.025	531	1.243	768

* signifikant zur supplementierten Gruppe, p < 0,05

Zusammenfassung

In zwei Legehennenversuchen mit und ohne Vitamin B₁₂-Zusatz im Futter waren die zootecnischen Leistungen im Mittel der Versuche nicht signifikant verschieden. Die Bruchfestigkeit und Dichte der Eischale sowie die Haugh-Einheiten lagen bei Futter ohne B₁₂-Supplementation signifikant höher als in der Kontrollgruppe mit B₁₂-Ergänzung. Die B₁₂-Konzentration des essbaren Eies und der Leber waren bei Futter mit B₁₂-Zusatz signifikant angestiegen. Auch die Exkremente der supplementierten Hennen enthielten mehr B₁₂. Die Konzentration von B₁₂ im Caecuminhalt war sehr hoch.

Summary

Vegetable diets without and with vitamin B12 supplements in laying hens

On the average of two experiments the vitamin B12 supplementation of vegetable diet of laying hens did not significantly take effect on zootechnical parameters. The shell breackage strength, the shell density, and the Haugh-units were significantly enhanced in the groups without vitamin B12 supplementation. Furthermore, the vitamin B12 concentration was significantly increased in egg contents and liver in the supplemented groups. This tendency was also observed in the vitamin B12 concentration of the excrements. The vitamin B12 concentration of the caecum content was very high.

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Blood vitamin concentrations in dogs before and after intake of diets with specified vitamin contents

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Introduction

The vitamin supply in companion animals is generally considered to be high, but data concerning the current situation are not available. There are only few studies on blood vitamin levels in relation to dietary vitamin intake of dogs. BAKER et al. (1986) presented a rather complete survey on the blood vitamin concentrations of healthy dogs, without reporting the vitamin intake. Other studies have focused on specific aspects of vitamin concentrations in blood samples of dogs. The purpose of this study was to give a survey of blood vitamin concentrations in healthy dogs and to study the impact of defined dietary vitamin intakes on blood vitamin levels and hair and skin quality.

Material and Methods

Dogs – Sixty-four healthy, privately owned healthy dogs (1 - 8 years) were included in a randomized double blind study. Dogs were randomly assigned to four treatment groups according to sex and bodyweight receiving diets with different vitamin concentrations.

Diets – Before the study all dogs received commercial complete dry or wet diets according to the feeding habits of their owners. Supplements with impact on the vitamin status were stopped at least 14 days prior to the initiation of the study. After enrolment in one of the experimental groups, the dogs received one out of four dry diets with specifically adjusted vitamin levels for 122 days (table 1).

Table 1: Vitamin concentrations in the experimental diets fed to the dogs for 122 days

	Diet 1	Diet 2	Diet 3	Diet 4
	Vitamin concentrations per kg			
Vitamin A, IU	4320	5450	3720	3050
Vitamin D, IU	700	800	600	650
Vitamin E, mg	86	87	94	95
Ascorbic acid, mg	70	57	59	66
Vitamin B1, mg	6	16	17	17
Vitamin B2, mg	5	17	19	19
Vitamin B6, mg	6	20	15	16
Vitamin B12, mg	0,09	0,1	0,1	0,1
Pantothenate, mg	15	34	31	33
Niacin, mg	25	70	78	80
Folic acid, mg	0,6	2,7	3,6	4,7
Biotin, mg	0,3	0,6	1,3	2,6

The vitamins were added to the diets prior to the extrusion process. Vitamin levels were adjusted so that diet 1 had the lowest vitamin levels; diets 2, 3 and 4 had 2-3 fold increased concentrations of water soluble vitamins compared to diet 1, except ascorbic acid. Vitamin B₁₂ concentrations were kept constant in all diets. Diets 2, 3 and 4 had increased biotin concentrations when compared to diet 1 (0.6, 1.3 and 2.6 mg/kg diet vs. 0.3 mg/kg diet in diet 1). Dietary allowances were adjusted according to maintenance requirements in order to keep the body weights constant.

Traits - Blood vitamin levels were measured before the experiment (day 1) and after a feeding period of 122 days on the specific diet. The blood was collected in the morning after 12 hours fasting from the jugular vein. Retinol and retinyl esters and α -tocopherol were analyzed by HPLC with either ultraviolet or fluorescence detection. 25-hydroxycholecalciferol was determined by HPLC coupled with mass spectrometry and ascorbic acid fluorometrically after derivatisation. Thiamine pyrophosphate, riboflavin (with FMN and FAD) and pyridoxal-5'-phosphate were analyzed by HPLC and fluorescence detection with a commercial kit (Chromsystems Instrument & Chemicals Ltd., Munich, Germany). Cobalamin, folate, pantothenate and biotin were analyzed by microbiological methods.

Statistics - Results were analyzed by SPSS 11.5 computer program (SPSS 11.5[®] for Windows, SPSS GmbH Software, Munich, Germany). Vitamin levels were evaluated for differences between the groups using the H-test. Within group comparison at days 1 and 122 was done by Wilcoxon test. Probability values of P<0.05 were taken as significant.

Results and discussion

Data of the blood vitamin levels at days 0 and 122 are summarized in table 2. The retinol concentrations were reduced at the end of the experiment, in particular in group 3 ($P<0.01$). Alpha tocopherol concentrations declined in group 1 ($P<0.05$), serum cobalamin concentrations increased significantly in all groups during the study ($P<0.01$). Ascorbic acid, thiamine pyrophosphate and riboflavin concentrations were not affected by the different experimental diets.

Table 2: Vitamin concentrations in blood of the experimental dogs at days 1 and 122

		day 1	day 122			
		All groups	Group 1	Group 2	Group 3	Group 4
Retinol	µg/L	829	688*	690*	645*	747*
Retinyl palmitate	µg/L	863	847	825	898	901
Retinyl stearate	µg/L	2076	1962	2057	2205	2120
Alpha tocopherol	mg/L	24.4	20.5*	22.7	22.7	21.2
Ascorbic acid	mg/L	2.9	2.5	3.7	3.2	3.1
Thiamine pyropho.	µg/L	89.7	93.5	96.6	99	101
Riboflavin	µg/L	21.2	21.5	20.7	23.8	23
FAD	µg/L	343	313 ^a	308 ^a	333 ^{ab 1)}	358 ^b
Pyridoxal-5'-phosphate	µg/L	54.4	29.3 ^{a†}	64.2 ^c	58.8 ^{bc}	47.4 ^b
Cobalamin	ng/L	869	1202*	1298*	1287*	1379*
Folate	µg/L	2.3	2.9	2.5	2.8*	2.9
Pantothenate	µg/L	308	345	372*	381*	420*
Biotin	ng/L	1618	1469 ^a	1548 ^a	2733 ^b	4911 ^{c†}

Means of groups with different superscripts in the same row differ significantly from each other, asterisks indicate significant changes between days 1 and 122 for the individual group ($p<0.05$); ¹⁾ Significant reduction compared to the group mean at day 1

The concentrations of FAD in group 3 were reduced compared to the baseline levels of this group ($P<0.05$), pyridoxal-5'-phosphate was lower in group 1 at day 122 ($p<0.001$), but did not change in the other groups. The blood levels of pantothenate increased in groups 2, 3 ($P<0.05$) and 4 ($P<0.01$). Folate increased only in group 3, the concentrations of biotin increased in all groups in strict correlation to dietary intakes ($r^2 = 0.98$; $y = 1218e0.548x$), however, this was only significant in group 4. In conclusion, this study gives a survey of blood vitamin concentrations in healthy dogs and provides a data base for the evaluation of the vitamin status in health and disease. Comparing the blood vitamin concentrations before and after the intake of the

defined diets allows the conclusion that the intakes of some vitamins were lower under field conditions, however, the intake of most vitamins seems to have exceeded the levels in the experimental diets.

Summary

The objective was to investigate blood vitamin concentrations in healthy dogs and to determine the effects of defined dietary vitamin intakes on blood vitamin levels. The dogs were assigned to four groups with different vitamin concentrations, adjusted to meet or exceed the dietary requirements. Diet 1 had the lowest analyzed vitamin levels; diets 2, 3, and 4 had 2-3 fold increased concentrations of water soluble vitamins when compared to diet 1. The blood vitamin levels of the dogs were investigated at day 0 and after the feeding of diets with specified vitamin levels at day 122. This study gives a survey of blood vitamin concentrations in healthy dogs and provides a data base for the evaluation of the vitamin status in health and disease. Comparing the blood vitamin concentrations before and after the intake of the defined diets allows the conclusion, that the dietary intakes of some vitamins were lower under field conditions, however, the intake of most other vitamins seems to have exceeded the levels in the experimental diets.

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Effect of baking and freezer storage time on added folic acid stability in wheat and rye breads

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Introduction

Folates are polyglutamates (usually 5-7 glutamyl residues) of pteronic acid and has been identified as one of the most important vitamin for normal human metabolic function. Folate nutritive is associated with neural tube defects (NTD) in newborns (Stevenson et al., 2000; Czeizel et al., 1992), cardiovascular diseases (Bousey et al., 1995), colon cancer (Rampersaud et al., 2002) and certain anemia (Fishman et al., 2000). Since humans cannot synthesize folates, they must obtain them from dietary sources. To reduce the risk of woman of childbearing age to have a child with neural tube defect, some countries mandated folate fortification of staple food. Folic acid is the monoglutamate of pteronic acid and is not a natural physiological form of the vitamin but is used by food processors to fortify food.

The objective of this study was to examine the effect of baking process on added folic acid and evaluate its stability during bread storage at -20°C.

Materials and Methods

Wheat (T-750) and rye (T-720) flours were purchased from the local Baker Factory and baker's yeast (polish standard PN -A-79002:1998) from the local market. Breads were produced using the formulation containing enriched wheat and rye flours with 0.4 mg folic acid/100 g flour and baker's yeast. The amount of doughs for bread making were 250 g, baking temp. 230-235°C, and time of 30 i 40 min. for wheat and rye breads, respectively. Breads were frozen at -20°C and held for up to 16 weeks. Fresh breads and breads frozen for 5, 10 and 16 weeks were measured for folic acid content. Samples were freeze-dried before analysis.

α-Amylase (EC 3.2.1.1, A-6211) and *protease* (EC 3.4.24.31, P-5147) were purchased from Sigma Chemical Co. and dissolved in Wilson and Horne extraction

buffer (Wilson and Horne, 1884) at the concentration of 20 mg/ml and 2 mg/ml, respectively. Folate conjugase (EC 3.4.22.12) from kidney, porcine, type II (Sigma, K-7250) was prepared according to Phillips and Wright (1983), isolated and purified by the method of Gregory et al. (1984). Folic acid (Sigma) was added to flour at a concentration of 300 µg/100 g.

All samples preparation was made under subdued light. 2 g of sample was accurately weigh into 50 mL Oak Ridge PPCO centrifuge tube (Nalgene Co.). 20 ml of Hepes/Ches buffer, pH 7.85 (50 mM Hepes, 50 mM Ches) containing 2% sodium ascorbate and 0.2M 2-mercaptoethanol were added to samples and homogenized using an Ultra Turrax T-25 homogenizer with dispersing elements S 25-18 G (IKA-Germany) for 30 s at a speed of 13500 rpm. Dispersing element was rinsed with 5 mL of extraction buffer. After deaerated of homogenized samples by flashing with nitrogen and capped tubes immediately with stoppers, the tubes were placed for 10 min. in a boiling water bath. Tubes were swirl occasionally during heat treatment, homogenized again, deaerated and cool immediately in ice. The homogenate was subjected to trienzyme treatment: 3 mL kidney folate conjugase, 1 ml α-amylase (pH = 4.9 adjusted with acetic acid, 4 h at 37°C) followed by 4 ml protease (pH = 7.0, 1 h at 37°C). After each treatment tubes were flashing with nitrogen and capped tightly with stoppers. The incubated extract was heated for 5 min in boiling water bath, cooled in ice and centrifuged (20 min at 12000 rpm, at 4°C). The residue was resuspended in ca 4 mL Hepes-Ches extraction buffer, centrifuged again and combined supernatants were filled to an exact volumes (25 or 50 ml), filtered through a Whatman filter paper No. 1, then flushed with nitrogen, and stored at – 80 °C until cleanup (usually a few days).

Sample clean-up

Purification of sample extract was carried out by solid-phase extraction on strong anion exchange (SAX, Baker 7091-03, 500 mg) according to Jastrebova et al. (2003).

HPLC analysis

The Phenomenex LunaC18 column (5 µm, 250 x 4.6 mm) was used for separation. The chromatographic condition for gradient elution were as follows: flow rate, 1 ml/min; volume injected, 1 ml; column temperature, 23°C; UV detection, 290 nm. Gradient elution was performed with acetonitrile-33 mM phosphate buffer, pH 2.3 (Pfeiffer et al., 19970). The run time was 30 min and the time between injections 42

min. The gradient was started at 5% (v/v) acetonitrile, maintained isocratically for the first 8 min, thereafter the acetonitrile concentration was raised linearly to 17,5% (v/v) within 25 min. Quantification was based on external calibration standard. Standard was prepared as described by Konings (1999), and concentration was calculated using molar absorption coefficients given by Blakley (1969).

Results

The results indicate that during the process of bread baking, the content of folic acid decreased by about 15% both in the wheat and rye breads. Storage of wheat and rye breads at -20°C for 5 weeks did not have a significant effect ($p < 0.05$) of folic acid compared to the content directly after baking. After a 10 weeks storage period, a significant decrease in the content of folic acid was recorded and it dropped on average by 18 and 17%, respectively, for the wheat and rye breads. After longer period of storage (16 weeks) an almost 33% loss of folic acid in the wheat bread and a 40% loss in the rye bread was found.

Table 1. Folic acid content ($\mu\text{g}/100\text{g}$ dry weight) of fortified flour, fresh breads and breads after freezer storage in -20°C.

Product	Folic acid ($\mu\text{g}/100\text{g}$ dry weight) ¹	
	Rye flour (T-720)	Wheat flour (T-750)
Fortified flour	348	348
Bread – fresh	303 \pm 22 ^{a/2}	296 \pm 15 ^a
Bread – after 5 weeks	301 \pm 28 ^a	282 \pm 30 ^a
Bread – after 10 weeks	252 \pm 13 ^b	242 \pm 9 ^b
Bread – after 16 weeks	183 \pm 16 ^c	199 \pm 19 ^c

1 – values are the mean \pm standard deviation of three replicate baking process

2 – in columns, means followed by different letters are significantly different ($p < 0.05$)

Summary

Studies were carried out to determine the effect of parameters applied to baking bread from wheat and rye flour on the loss of the added folic acid and to evaluate its stability during bread storage at -20 C. The content of folic acid was measured directly after baking and 5, 10 and 16 weeks after storage with the use of high performance liquid chromatography HPLC. The results indicate that the folic acid exhibits a relatively high stability and can be successfully added to flour designated for bread which, after baking, is to be stored at -20 C for 10 weeks.

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Determination of B vitamins in infant milk by high-performance liquid chromatography.

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Introduction

The water-soluble B vitamins are group of organic compounds that are essential for good health and growth of the child. This work describes the high-performance liquid chromatographic (HPLC) techniques, which allow rapid separation, qualification and quantification of B vitamins in infant foods using reversed-phase columns and UV detection. In this study, the method was applied to determine vitamins: B₁ (thiamine), B₂ (riboflavin), B₆ (pyridoxamine phosphate, pyridoxine hydrochloride, pyridoxamine dihydrochloride) in powdered infant milks. The use of the certified references materials allowed a good validation of the proposed method.

Materials and methods

Standards

The vitamin standards were obtained from Sigma- Aldrich (St. Louis, MO, USA). Stock standards were prepared in distilled water in order to obtain calibrated solutions containing: 2.0, 1.0, 0.5, 0.2, 0.1, 0.05, 0.01, 0.005 $\mu\text{g ml}^{-1}$ for the all vitamins. All solutions were stored at 4⁰C in glass light-resistant bottle. The working solutions were prepared daily and passed through a 0.22 μm pore-size filter before being injected into the column.

Samples and Certified Reference Materials

In a first collaborative study, we used seven one batch powdered milks samples. They were supplemented with vitamins and purchased in local supermarket. In a second collaborative study, we used seven different types of powdered infant milk (starting milk, follow-up milk) deriving from two production date. The method was validated using the certified references material (milk powder CRM 421), which was supplied by the Community Bureau of Reference, BCR (Belgium). The samples (5g of powdered milks and powdered infant milks, 2.5 g certified references material) were finely ground. Samples were submitted to hydrolysis according to procedure described by Ndaw et al. (2000). The certified reference samples were analyzed in the same way. For statistical analysis the Student's t-test described by Szczepaniak (2002) was used.

Reagents

Enzymes used: taka-diastrase from *Aspergillus oryzae* (Fluka, catalogue No.86247), acid phosphatase from potato type II (Sigma-Aldrich, catalogue No.P3752).

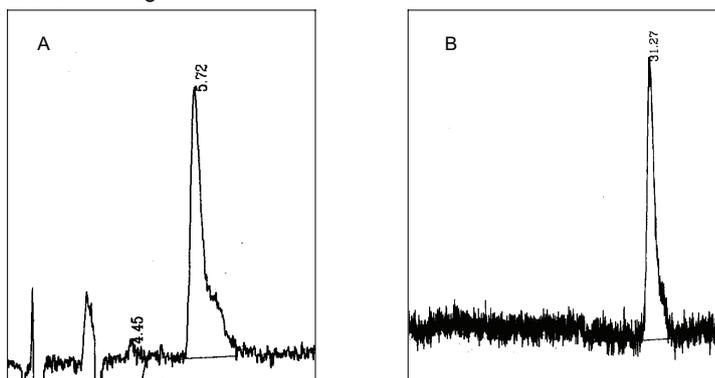
Acetonitrile (for HPLC) and all other chemicals from Sigma- Aldrich (St. Louis, MO, USA) and from Merck (Darmstadt, Germany).

Chromatographic determination

Chromatographic separations of all vitamins were performed on a reversed-phase C₁₈ column Lichrospher RP 18 (5.0 µm particle size, 250x4.6mm i.d.). Separation by ion pair chromatography was accomplished isocratically with acetonitrile/0,05M potassium dihydrogen phosphate (10:90, v/v) containing 0,3 x 10⁻³M sodium octane sulfonate as mobile phase. The mobile phase was then adjusted to pH 2 with orthophosphoric acid. The separation was performed at 30⁰C at a flow rate of 1ml min⁻¹, the UV-detection at 260 nm for thiamine hydrochloride, 268 nm for riboflavin and 290 nm for vitamin B₆ (pyridoxamine phosphate, pyridoxine hydrochloride, pyridoxamine dihydrochloride). The injection volume was 50µl. All five compounds were separated in less then 35 min. Peaks identification were based on the retention time, by comparison of the ratio of UV spectra with that of standard commercial compounds. The conditions of separation were those described by Arella et al. (1996) and Bergaentzlé et al. (1995).

Results

The B group vitamins are soluble in water and have an ionic character, making them suitable for reversed phase chromatography. A detailed study on the influence of the ACN/buffer percentage proportion, flow-rate, temperature and pH was performed. This HPLC method proposed in the collaborative studies for vitamin B₁, B₂ and B₆ determination was the method described by Arella et al. (1996) and Bergaentzlé et al. (1995) with only minor modifications. The effect of the best chromatographic separations was obtained at slight modification of acetonitrile/0,05M potassium dihydrogen phosphate proportion of the mobile phase (10:90, v/v) instead (4:96, v/v) and with adjusting to pH 2.0 instead to pH 2.5. The separation pattern of the vitamins is shown in Fig. 1.



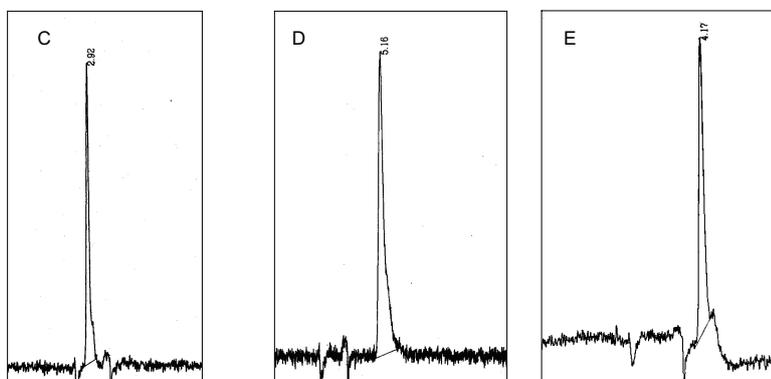


Figure 1. Chromatogram of standards: (A) B₁ (thiamine), $t_R = 5.72$ min; (B) B₂ (riboflavin), $t_R = 31.27$ min; (C) B₆ (pyridoxamine phosphate), $t_R = 2.92$ min; (D) B₆ (pyridoxine hydrochloride), $t_R = 5.16$ min; (E) B₆ (pyridoxamine dihydrochloride), $t_R = 4.17$ min.

The linearity of standard curves (Tab. 1) was expressed in terms of correlation coefficient (R), from plots of the integrated peak area (% of UV spectra) vs. concentration of the standard ($\mu\text{g ml}^{-1}$). A linear equation was found, with satisfactory linearity ($R > 0.99$).

Table 1. Calibration characteristics of B vitamins

Vitamin	Powdered milk (supplemented with vitamins) ($\mu\text{g g}^{-1}$)		Linearity test			Recovery test (Milk powder CRM 421)		
	Mean \pm SD ^a	RSD ^b (%)	Linear range ($\mu\text{g ml}^{-1}$)	R ^c	LOD ^d ($\mu\text{g ml}^{-1}$)	Fund ^e ($\mu\text{g g}^{-1}$)	Certified ^f ($\mu\text{g g}^{-1}$)	Recovery (%)
B ₁	8.38 \pm 0.16	1.85	0.01-2.0	0.9999	0.01	6.39 \pm 0.09	6.51 \pm 0.48	97.8
B ₂	8.80 \pm 0.20	2.22	0.01-2.0	0.9999	0.01	14.16 \pm 0.20	14.5 \pm 0.60	97.6
B ₆ ^h	3.49 \pm 0.09	2.64	0.01-2.0	0.9999	0.01	-	-	-
B ₆ ⁱ	16.97 \pm 1.24	7.31	0.01-2.0	0.9999	0.01	-	-	-
B ₆ ^j	7.79 \pm 0.16	2.09	0.005-2.0	0.9998	0.005	-	-	-
B ₆ (total) ^g	26.10 \pm 1.23	4.70	-	-	-	5.46 \pm 0.19	6.66 \pm 0.85	82.2

^a Mean \pm standard deviation, n=7.; ^b Relative standard deviation, n=7.; ^c Correlation coefficient.; ^d Limit of detection.; ^e Mean \pm standard deviation, n=3.; ^f Certified values.; ^g The total pyridoxamine phosphate + pyridoxine hydrochloride + pyridoxamine dihydrochloride expressed as pyridoxine hydrochloride; ^{h, i, j} Pyridoxamine phosphate, pyridoxine hydrochloride, pyridoxamine dihydrochloride, respectively.

The precision of the method was demonstrated by repetitive analyses, calculating the average relative standard deviation (RSD) for the determination of concentration of these all vitamins in seven one batch samples of powdered milks (Tab. 1). The

relative standard deviations obtained for all vitamins were less than 10%, which is the normal level in any food quality control and being always satisfactory according to the Horwitz's formula for intra-laboratory analysis (Horwitz et al. 1998).

Validation was performed using the certified references materials (milk powder CRM 421). The results obtained are shown in table 1. The recovery were: 97.8%, 97.6% and 82.2% for vitamin B₁, B₂, B₆ (expressed as pyridoxine hydrochloride), respectively. For both vitamins B₁ and B₂ the recovery rates of the method were over all satisfactory for the determination of these vitamins in the powdered milk. For determination of vitamin B₆ the value was not entirely satisfactory.

This chromatographic procedure was applied to the determination of the B group vitamins in the different powdered infant milks. These results are show in table 2.

Table 2. Determination of B group vitamins in various powdered infant milks

Vitamin	Concentration ^a (µg g ⁻¹)						
	Infant milk						
	Starting from 1 to 5 month	Starting from the born	Fallow-up from 5 month	Fallow-up above 4 month	Fallow-up above 12 month	Fallow-up from 1 to 3 year	Fallow-up from 1 to 3 year (with honey)
B ₁	6.3±0.2	5.2±0.1	10.3±0.2	9.9±0.2	6.8±0.1	10.3±0.2	16.3±0.2
B ₂	9.0±0.5	7.6±0.3	13.5±0.5	7.5±0.3	11.2±0.3	13.9±0.3	19.1±0.5
B ₆ (total)	5.2±0.1	4.8±0.1	12.1±0.2	5.3±0.1	9.6±0.2	18.8±0.3	19.6±0.3

^a Mean ± standard deviation, n=3.

All five compounds were separated in less then 35 min. All vitamins were identified in any of the infant milk samples above the detection limits. The procedure was applied for determination of the vitamins in infant foods with very good results.

Summary

The method proposed to determine B vitamins in powdered infant milk yields satisfactory results for powdered samples. The method proposed is fast and offers satisfactory specificity, precision and accuracy. Linearity, recovery and sensitivity were satisfactory. The simplicity of the procedure should make it highly desirable for quality control of baby powdered foods.

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Analysis of pantothenic acid and folic acid: conclusions from method comparisons

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Introduction

(R)-Pantothenic acid (PA), on the one hand, is a vitamin occurring in foods and feed-stuff in its free form as well as in conjugates such as coenzyme A (CoA) or acyl carrier protein (ACP), which are involved in metabolism of steroids, fatty acids and phosphatides.

On the other hand, vitamins of the folate group function as physiological methyl donors and cofactors involved in DNA synthesis. In this context, folate deficiency is supposed to prevent neural tube defects, alzheimer's disease and cardio vascular disease. Furthermore, folate deficiency appears to be the cause for the meanwhile evident correlation between the very frequent low dietary intake of folates and the risk of cancer. For this reason, fortification of staple foods became mandatory in several countries, particularly on the american continent. Therefore, data on dietary uptake of PA and folates by humans as well as by cattle are important. Notwithstanding, the instrumental developments in chromatography and mass spectrometry, vitamin analysis has still remained a challenge. Lability, trace concentrations and bound forms are major roadblocks for vitamin quantitations as reviewed recently (Rychlik and Köhler, 2005). Actual developments are the use of labelled PA and folates as internal standards (IS) in stable isotope dilution analysis (SIDA). However, direct comparisons of SIDA with alternative method are still lacking. Therefore, the objective of the present study was to perform a site-by-site comparison of identical samples analyzed by different assays.

Material and Methods

[¹⁵N, ¹³C₃]-pantothenic acid (Rychlik, 2000) and [²H₄]-folic acid (Freisleben et al., 2003) were synthesized as reported recently.

Cereals were ground in a grain mill (Bosch, München, Germany), cereal products and hazelnuts were minced in a blender (Privileg, Quelle, Fürth). Porcine liver, whole eggs and mushrooms were lyophilized and the residue ground in a mortar.

For analysis of PA, the resulting powders (2 g) were stirred for 24 hours at 20 °C in an aqueous solution of sodium acetate containing papain and diastase. Each extract was then filtered and divided into two halves, one of which was used for SIDA and the other for MA.

SIDA for quantification of free and total pantothenic acid. To an aliquot (10 mL) of each extract, calcium [¹⁵N, ¹³C₃]-(*R*)-pantothenate (5 µg) was added and the resulting solution, after passing through a syringe filter (0.4 µm, Millipore, Bedford, MA, USA), was analyzed for free PA by LC/MS/MS. Enzymatic hydrolysis of PA conjugates was performed as reported recently (Rychlik, 2003).

Microbiological assay for PA. The extracts were assayed for pantothenic acid with *Lactobacillus plantarum* (ATTC 8014) .

SIDA for FA. For analysis of FA in fortified cereal products, the resulting powders or flours (0.5 g) were stirred for one hour at 20 °C in extraction buffer containing [²H₄]-folic acid (400 ng). The extracts were filtered and, after passing through a syringe filter (0.4 µm, Millipore, Bedford, MA, USA), analyzed by LC/MS/MS as detailed recently.

HPLC-UV for FA were performed according to a procedure reported recently (Krishnan et al., 2005). In short, samples (2g) were agitated in pH 11 buffer for one hour, filtered , and cleaned up by solid phase extraction prior to injection on a LichrosorbC18 HPLC column, which was run with a gradient consisting of 10 to 30% acetonitrile in 33mM phosphoric acid buffer, and UV detection at 285nm.

Results and Discussion

Method comparison for PA

For sample preparation we employed a standard procedure described by Roth-Maier et al. (2000). Sample extracts were divided in two halves, of which the first half was analyzed by monitoring spectrophotometrically the growth of *Lactobacillus plantarum*. To the second half of the extract, the isotopomeric standard [¹⁵N, ¹³C₃]-PA was added and the solution was analyzed by LC-tandem mass spectrometry. As SIDA without further enzyme treatment cannot measure PA conjugates such as coenzyme A or

acyl carrier protein, aliquots of the solutions additionally were incubated with pantetheinase and phosphatase.

Table 1 Comparison of pantothenic acid (PA) data obtained by stable isotope dilution assay (SIDA) and by microbiological assay (MA) with those reported in the literature

mg/100 g sample	SIDA of free PA	total PA	MA	Literature (Souci et USDA, 2000 ; 2001)	data al., 2000 ; 2001)
millet	0.37	0.61	0.76	0.35	— 1.20
wheat	0.64	0.84	1.07	0.85	— 1.75
corn	0.22	0.65	0.65	0.48	— 0.76
potato	0.25	0.25	0.28	0.26	— 0.50
cocoa	0.67	1.39	1.36	0.2	— 1.1
hazelnuts	0.85	1.22	1.64	0.918	— 1.18
soy beans	1.45	1.76	1.91	0.80	— 1.95
orange juice	0.08	0.08	0.18	0.15	— 0.17
mushroom	1.17	1.29	1.23	1.5	— 2.7
whole egg pwd.	7.78	8.09	9.53	5.905	— 10.0
skim. milk pwd.	5.23	5.61	6.33	3.39	— 3.50
porcine liver	4.35	5.23	6.59	6.4	— 7.0

PA in feeds and foods of plant origin

The results of plant-derived samples are listed and compared to the data of data bases in table 1. For cereals, potatoes, cocoa, corn and mushrooms, the results of the microbiological assay (MA) and SIDA of total PA were in quite good accordance with a maximum deviation of 21 % for wheat. Moreover, our results were quite well in line with the two data bases considered. However, the situation was quite different for the remaining samples derived from plants. For hazelnuts, a significant difference of 25 % between MA and SIDA was observed. The reasons for this discrepancy remain open, but the data imply that nuts contain substances, which stimulate the growth of the microorganisms without being measured by SIDA, even after all enzyme treatments. These compounds might be yet unknown PA forms or substances that the microorganisms can utilize for their growth and that are structurally different from PA conjugates. This might also hold true of millet, wheat and orange juice, for which the MA gave significantly higher PA figures than SIDA.

PA in feeds and foods of animal origin

Whole-egg powder, skimmed milk powder and porcine liver were also analyzed by the two methods and revealed much more PA contents than the foods and feeds of plant origin. In analogy with the latter products, PA data of SIDA were lower than those of MA.

Table 2 Comparison of folic acid data obtained by stable isotope dilution assay (SIDA) and by HPLC-UV in a collaborative study (CS) and by different extraction conditions

$\mu\text{g/g}$	Sample	Label	SIDA	Mean	HPLC	HPLC
Breakfast cereals	Rice Krispies	3.03	2.82 ^a	3.32	2.32 ^b	2.98 ^c
	Krispies	9.66	9.65 ^{ab}	7.87	10.19 ^a	9.13 ^b
	Krispies	8.48	9.84 ^{ab}	4.80	10.13 ^a	8.59 ^b
	Corn Flakes	3.57	6.30 ^a	5.88	5.43 ^a	5.43 ^a
	Wheat pops	6.66	7.42 ^a	5.92	7.46 ^a	5.75 ^b
	Wheat rings	6.66	6.55 ^a	5.61	10.45 ^b	4.34 ^c
	Wheat bars	1.00	1.23 ^a	1.32	1.26 ^a	0.70 ^b
Noodles	Maccaroni	1.79	1.62 ^a	2.16	1.49 ^a	2.98 ^b
Cookies	Cookies	1.33	0.81 ^a	0.94	1.45 ^b	1.42 ^b
	Whole wheat	13.3	12.39 ^a	11.49	12.69 ^a	8.30 ^b
	Crackers	2.67	1.35 ^a	1.56	2.23 ^b	1.63 ^a
Flours	Corn meal	1.48	1.93 ^a	2.15	1.94 ^a	1.43 ^b
	Wheat flour	1.33	1.63 ^a	1.22	0.93 ^b	1.03 ^b
Bread	Wheat bread	0.88	1.38 ^a	1.62	1.45 ^a	1.99 ^b
Infant	formula	1.29	1.18 ^a	0.73	1.07 ^a	1.64 ^a

Means with the same letter within rows are not statistically significantly different ($P \geq 0.05$).

Total PA measurement by MA and SIDA

As noted above, MA nearly for all foods and feedstuffs gives somewhat higher data than SIDA of total PA. This result indicates that MA data using diastase and papain include conjugated PA without the need for further enzymatic treatment.

FA measurement by HPLC-UV and SIDA

In 2001, a multi-laboratory evaluation of HPLC folic acid methods applied to several cereal samples revealed considerable variability in folic acid data. A closer look at the data showed that laboratories that employed simple aqueous extraction techniques showed good precision between blind duplicates and recovered folic acid values closer to values claimed in the food labels. As seen in table 2, an alkaline pH of

11 and agitation using a stir-bar favored the dissolution of folic acid from sample matrices and yielded results that were in close agreement with LCMSMS values for the same samples. Use of large capacity SPE devices (6mL capacity) ensured optimal retention of PGA on SPE cartridges and recovery of added folic acid from most of the cereal matrices tested. Blind duplicates embedded within the samples yielded excellent precision. Aqueous pH 11 extraction yielded FA values for 10 samples that were statistically not different than those analyzed by SIDA. Some samples showed anomalous and high FA values with alkaline pH and this was not observed with extraction at pH 7.85. The use of α amylase and protease enzymes traditionally used in sample preparations did not provide advantages in the analysis of added folic acid in cereals samples.

Conclusions

The method comparison presented here revealed for a number of foods and feedstuffs a quite good accordance between the MA and the SIDA of total PA. From this finding and from the results of the different enzyme treatments it can be concluded that MA is able to measure a significant amount of bound PA. Moreover, MA showed significantly higher data than SIDA of total PA, which could only be explained by the presence of compounds that show microbiological PA activity, but do not liberate PA when treated with common deconjugating enzymes.

For FA, SIDA was used to obtain the "true" content and served to optimize the extraction procedure for HPLC-UV analysis, which thereafter yielded quite accurate results.

Summary

Method comparisons were performed for the quantitation of pantothenic acid and folic acid between a stable isotope dilution assay (SIDA) using [$^{13}\text{C}_3$, ^{15}N]-pantothenic acid and [$^2\text{H}_4$]-folic acid as the internal standards and a microbiological assay and an HPLC-UV method, respectively.

For pantothenic acid (PA), different foods and feedstuffs were analyzed by the recently developed SIDA and by the standard method, a microbiological assay (MA). For samples derived from plants a good accordance between the MA and the SIDA of total PA was found, whereas for the products of animal origin higher contents were measured by MA than by SIDA. Furthermore, the data imply that microbial enzymes

were able to cleave PA conjugates more effectively than pantetheinase and phosphatase treatment.

Although significant process in terms of precision and accuracy has been achieved by using stable isotope dilution technology in enriched foods, for evaluations of the bioavailability of naturally occurring vitamins, microbiological assays may still be of use.

For folic acid (FA) in enriched cereal products, SIDA resulted in data quite close to the labelled amounts of FA. Further experiments revealed that extraction at pH 11 and the use of large capacity SPE cartridges significantly improved the the HPLC-UV results.

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Impact of B₂ and B₆ supplementation of low protein diet on blood cells count and β -oxidation activity in lymphocytes of physically exercised rats.

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Introduction

Vitamins B₆ and B₂ are the source of coenzymes participating in single-carbon residues metabolism. The one-carbon units mainly originated from serine or glycine are transferred to 5-methyltetrahydrofolate. In this reaction methionine is formed which may be converted to universal methyl groups donor S-adenosylmethionine. It was noticed that n-3 fatty acids with oleic acid and vitamin (E, B₆ and folic acid) supplementation decrease plasma LDL cholesterol and homocysteine concentration what may have favourable effects on risk reduction of coronary heart disease (Baro et al., 2003). Vitamin B₆ is known to be necessary for stability and physiological function of cells membranes. Chang (2000) found in mice given B₆ antagonist, that vitamin B deficiency caused ultrastructure abnormalities of arterial endothelial cells. Many experimental studies show close link between vitamin B₆ content in the diet and metabolism of PUFA. Witten and Holman (1952) demonstrated that B₆ deficiency may affect transformation of linoleic acid to arachidonic acid. They observed a decrease of arachidonic acid concentration in tissues of rats fed B₆ deficient diet. This observation was confirmed by Goswami and Coniglio (1986) and Bergami et al. (1999). During a period of B₆ deficiency a simultaneous decrease of linoleic acid concentration and an increase of other polyunsaturated acids in rat's testis were observed.

In previous study Bertrandt et al. (2004) have shown that decrease of energy originated from protein from 20% to 9% did not change hepatic fatty acid composition during 3 months of experimental treatment, however in serum decrease of arachidonic and docosahexaenoic acids was observed. The enrichment of such low protein diet with vitamin B₆ caused a decrease of linoleic acid and increase of α -linolenic and docosahexaenoic acids in blood serum. These results suggest that dietary supplementation with vitamin B₆ may stimulate pathways of PUFAs' synthesis during long lasting dietary protein deprivation. Observed changes of fatty acids amount and composition occurring with simultaneous vitamin concentration changes may influence production of energy in the cells.

Manore (2000) stated that exercise riboflavin requirements appear to increase in with physical exercise as well as increased urinary excretion of B₆ metabolites. It seems that active individuals given low protein diet might face increased risk of poor status of these vitamins. This observation is very important because these vitamins are cofactors for many metabolic reactions producing energy in cells. To study this influence the estimation of β -oxidation efficiency in separated leucocytes was chosen.

The aim of this experiment was to study the effect of vit. B₂ and B₆ diet enrichment on blood cells composition and β -oxidation efficiency in isolated lymphocytes of physically exercised rats fed protein deficient diets.

Materials and methods

The growing male Wistar rats initially weighting 144.5 ± 2.5 g were kept in individual stainless steel cages in room temperature with controlled, humidity and lighting (12h day cycle). In this experiment rats were fed *ad libitum* semisynthetic isocaloric diets of 1466.5 kJ/100g (350kcal/100g) throughout 90 days. In standard diet 20% energy was provided from protein, 15% from fats, including 2% from essential fatty acids (control diet). Diet deficient in protein was also used – in this diet only 9% of energy was provided from protein utilization. Half of animals receiving protein deficient diets were supplemented with 4.5 mg vitamin B₆ to the level 4-times higher than in the control diet. Twenty rats were used per treatment. Sunflower oil was the source of essential fatty acids. Rats were running using horizontal runway for 1 hour daily at speed of 20 m. per min., 5 days a week. 12 h prior to the end of experiment food was withheld. Rats were sacrificed by cervical displacement while unconscious (general anesthesia using ethylene ether) and blood was taken by hart puncture using standard EDTA as anticoagulant. Lymphocytes were isolated by centrifugation on gradient of 3 ml of HISTOPAQUE–1077 (Sigma). Turbid interphase layer of lymphocytes was collected, diluted with 5 ml of PBS and recentrifuged. This step was repeated 3-times. Final pellet of lymphocytes was resuspended in PBS and used for experiments. Substrate was prepared from [9,10]-³H palmitic acid and non labeling palmitic acid in Hank's balanced salt solution (HBSS) contained 0.5 mg/ml of fatty acids free bovine albumin (BSA) at final concentration of 52,4 pmole of substrate in reaction mixture. Samples were incubated in triplicate. The activity was measured as the amount of tritiated water formed and expressed as pmoles of substrate degraded by 1 mg of lymphocyte protein in 1 min. For statistical data ANOVA and median test were used. Differences of means at $P \leq 0.05$ (n=20) were considered statistically significant.

Results

In physically exercised rats receiving low protein diet statistically significant decrease of haematocrit value without red blood count changes, however with decrease of MCV and hemoglobin concentration in the blood. In these blood samples lower white blood cells count was also observed. Low protein diet as well as used vitamin B supplementation did not affect platelets count. The efficiency of β -oxidation in lymphocytes of rats given low protein diet significantly decreases, however the enrichment of this diet with vitamin B₂ or B₆ counteracts the development of this change (table 1).

In the blood of rats supplemented with vitamin B₂ increased haematocrit value was observed. In the blood of animals supplemented with B₆ increased RBC count, hemoglobin concentration and haematocrit value was noticed.

Table 1: The influence of diet enrichment with vitamin B₂ or B₆ on blood indices of physically exercised rats fed 3 months with low protein diet. n=15

Parameter	20% energy from protein	9% energy from protein	9% energy from protein + B2	9% energy from protein + B6
RBC (T/l)	8,37±0,46 ^a	8,32±0,43 ^a	8,35±0,68 ^a	9,08±0,63 ^b
HCT (l/l)	41,68±1,45 ^b	38,49±2,72 ^a	41,06±1,75 ^b	43,19±2,13 ^c
MCV (fl)	49,89±2,09 ^b	47,38±2,26 ^a	47,73±1,75 ^a	47,5±2,24 ^a
HGB (g/l)	134,2±4,7 ^b	124,5±4,8 ^a	125,1±4,3 ^a	132,7±5,2 ^b
WBC (G/l)	4,82±0,70 ^b	4,46±0,91 ^a	4,13±0,71 ^a	4,46±0,63 ^a
PLT (G/l)	767,8±117,4 ^a	710±103,3 ^a	790,7±73,0 ^a	741,7±91,8 ^a
β -oxidation	43.54±4.26 ^a	36.79±2.78 ^c	40.22±3.75 ^{a,b}	41.82±2.69 ^a

Means \pm SD, Means not sharing a common superscript letter are statistically different (P<0.05)

Discussion

The question if certain vitamin supplementation can improve training performance especially in condition of low protein intakes is frequently asked. Vitamin B₆, in its active form pyridoxal 5'-phosphate (PLP), is a cofactor of many enzymes taking part in metabolic transformations of amino acids and nitrogen-containing compounds. Physical exercise stresses metabolic, pathways what is connected with use of riboflavine and vitamin B₆, so the requirement of both these vitamin may be elevated especially in case of long lasting use of poor diet. Capo-chichi et al., (2000), in protein-energy malnourished children, have shown significantly lower efficiency of riboflavin conversion into its cofactors leading to plasma FAD deficiency. The reduction of FAD dependant enzymes activity might influence energy production.

It is well known that exercise is capable to increase not only energy but also protein needs leading to several changes observed in trained organisms. Even of short

duration exercise are able to keep lower body weight of animals and reduce fat deposition, when rats were given high fat/carbohydrate diet (Kretschmer et al., 2005). Petradou et al., (2005) observed that 8 weeks of rats exercise training leads to significant differences in TG and FA composition of muscles and adipose tissue but not in the liver. In this experiment we have shown that energy production in lymphocytes of trained rats fed low protein diet is significantly decreased. It was also noticed that in animals fed low protein diet enriched with riboflavin or vitamin B₆ this inhibition of β -oxidation was not observed. This observation support the thesis that active animals and probably also humans have higher requirement of these vitamins. Physical training influence also blood paramethers of active animals. Kennedy et al. (2005) have observed that rats running wheels average 9,9 km/week posses elevated mean corpuscular content of hemoglobin as well as increased LDL/HDL ratio in the blood. They observed also differences in WBC composition in trained for 4 weeks rats. In our study we observed that feeding of trained rats with low protein diet caused decrease of hematocrite value, MCV and hemoglobin content. Enrichment of such low protein diet with vitamin B₆ counteracts appearance of these changes connected mainly with red blood cells content in the whole blood. White blood cells count as well as platelets content was not influenced by examined factors. Results obtained in this experiment suggest that animals fed protein deficient diet and subjected physical training possess increased risk of poor riboflovine and vitamin B₆ status and/or the efficiency of these vitamins transformation to active enzymes co-factors is diminished.

Summary

60 male Wistar rats, weighing 144.5 ± 2.5 g were randomly divided into for groups fed with the high protein diet delivered 20% of energy from protein (control) or with diets delivered 9% of energy from protein without vitamin supplementation (group 2) and supplemented with B₂ (group 3) or vitamin B₆ (group 4). Using horizontal runway rats were subjected to running for 1 hour daily at speed of 20 m. per min., 5 days weekly. The endurance training of the animals was performed for 3 subsequent months. Blood parameters i.e. RBC, HCT, MCV, HGB, WBC and PTL were examined, as well as fatty acids degradation in all groups of animals. It was no effect of any experimental variants on platelets count in the blood. RBC number was increased only in group 4 but their volume was the biggest in control animals. Low protein content in the diet without any vitamin supplementation caused decrease of haematocrit value and WBC count. Fatty acid utilization was highest in animals fed a standard diet (20% of protein). However, addition of vitamins activates increase β -oxidation in lymphocytes and B₆ was found to be a better activator of this metabolic pathway than B₂.

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The literature is available with the author.

Studies on the bioavailability of L-carnitine compounds

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Introduction

L-carnitine is essential for beta-oxidation of long chain fatty acids. Physical exercise or diseases such as ischemia, neuropathy, AIDS, or hemodialysis develop a need for additional L-carnitine [1-4]. To meet the requirement under these conditions, dietary supplements can be helpful. Base of free L-carnitine is highly water soluble; the absolute bioavailability is relatively low. It is largely unknown whether chemical modification of free base of L-carnitine into its salts or esters influences its absorption kinetics. Therefore, the aim of the present study was to compare absorption rate and kinetics of various L-carnitine esters (acetyl-L-carnitine and lauroyl-L-carnitine) and organic salts (L-carnitine L-tartrate, L-carnitine fumarate, L-carnitine magnesium citrate) in piglets as model animals.

Materials and Methods

Animals and treatment

Thirty eight male crossbred piglets with an average body weight of 13.3 ± 0.3 kg and ages of 6 to 8 weeks were used for this study. Twelve hours before the bioavailability test, the animals were deprived of food. Six groups of five or six piglets each were administered orally a single dose of 40 mg L-carnitine equivalents/kg body weight of each of those L-carnitine compounds. A seventh group served as a control. All carnitine compounds were supplied in powder form by Lonza GmbH, Wuppertal, Germany.

Sample collection

Blood samples were collected from *vena carotis communis* into heparinized tubes at 0, 1, 2, 3.5, 7, 24, and 32 hours after administration of carnitine supplement. Plasma was obtained by centrifugation of the blood at $1800 \times g$ for 10 minutes at 4°C. Plasma samples were stored at -18°C until analysis.

Carnitine analysis

The determination of the concentration of carnitine was based on procedures of Maeda and Stanley [5] as well as Wieland *et al.* [6] and was measured spectrophotometrically at a wavelength of 405 nm by a Spectrafluor plus plate reader (Tecan, Crailsheim, Germany).

Calculations and statistical analysis

The determination of the area under the curve (AUC) as calculated with the software Origin 5.0, Microcal. The relative bioavailability of the L-carnitine compounds (related to that of base of free L-carnitine) was calculated from the AUC values:

$$\text{Relative bioavailability} = (\text{AUC}_{\text{compound}} / \text{AUC}_{\text{Base of free L-carnitine}}) \times 100$$

Results

Concentrations of free and total L-carnitine and total L-carnitine in plasma

Single-dose administration of all the L-carnitine compounds caused a time-dependent increase of free and total carnitine in plasma (Figure 1 and 2). Peak concentrations and the time at which they were achieved, however, were different for the various compounds. At administration of L-carnitine L-tartrate, peak levels of free and total carnitine were already reached after 3.5 hours; at administration of all the other carnitine compounds, peak levels of free and total carnitine were reached after 7 hours. After reaching their peak level, the concentrations of free and total carnitine declined to the control level.

AUC values

The baseline corrected AUC values for free and total L-carnitine were not significantly different in pigs administered L-carnitine salt than in pigs administered L-carnitine ester, with the exception for free carnitine in plasma, calculated for the ranges between 0 and 32 (Table 1).

Relative bioavailability

The L-carnitine salts had a similar bioavailability as base of free L-carnitine, based on AUC values of both free and total plasma carnitine (Table 1). Bioavailability of L-carnitine esters was lower than those of base of free L-Carnitine and the L-carnitine salts.

Table 1: Baseline corrected areas under the curve (AUCs) for plasma free and total carnitine; Relative Bioavailability of various L-carnitine salts and L-carnitine esters (timeinterval between 0 and 32 hours)

Compound	Free base		L-carnitine salts			L-carnitine esters	
	L-carnitine	L-carnitine L-tartrate	L-carnitine fumarate	L-carnitine Magnesium citrate	Acetyl L-carnitine	Lauroyl L-carnitine	
	(6)	(6)	(6)	(5)	(5)	(6)	
Time interval	AUC (free carnitine, $\mu\text{mol} \cdot \text{h} \cdot \text{L}^{-1}$)						
0-2 hours	8 ± 10 _b	14 ± 9 _a	11 ± 6 _{ab}	7 ± 4 _b	8 ± 5 _b	6 ± 6 _b	
0-3.5 hours	18 ± 18	37 ± 13	36 ± 12	19 ± 8	19 ± 9	16 ± 12	
0-7 hours	57 ± 41 _a	82 ± 27 _a	63 ± 21 _a	55 ± 19 _a	49 ± 18 _b	43 ± 26 _b	
0-32 hours	269 ± 68	255 ± 67	255 ± 25	264 ± 70	166 ± 64	159 ± 45	
Time interval	AUC (total carnitine, $\mu\text{mol} \cdot \text{h} \cdot \text{L}^{-1}$)						
0-2 hours	17 ± 8 _a	18 ± 11 _a	14 ± 9 _{ab}	8 ± 8 _b	9 ± 7 _{ab}	4 ± 7 _b	
0-3.5 hours	36 ± 18 _a	41 ± 16 _a	30 ± 14 _{ab}	21 ± 14 _b	21 ± 13 _{ab}	16 ± 12 _b	
0-7 hours	85 ± 44 _a	98 ± 31 _a	76 ± 23 _{ab}	56 ± 23 _{ab}	58 ± 25 _b	46 ± 25 _{ab}	
0-32 hours	288 ± 72 _{ab}	307 ± 129 _a	301 ± 63 _{ab}	241 ± 74 _{ab}	190 ± 135 _b	201 ± 65 _{ab}	
Base (AUC of)	Bioavailability (% , relative to Base of free L-carnitine)						
Free carnitine		92 ± 19 _a	93 ± 9 _a	84 ± 17 _a	61 ± 14 _b	43 ± 16 _b	
Totale carnitine		106 ± 20 _a	104 ± 13 _a	89 ± 15 _{ab}	66 ± 15 _b	70 ± 26 _b	

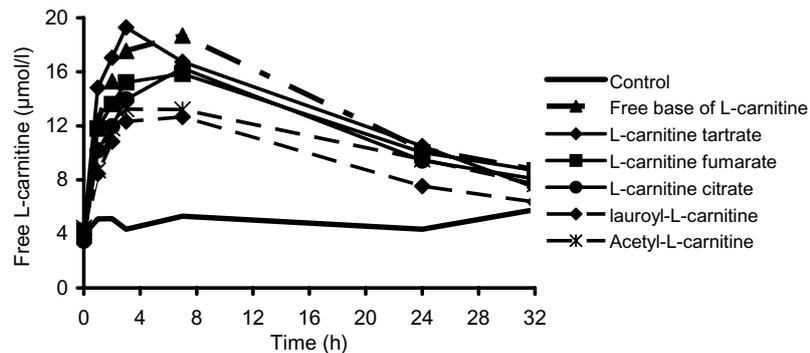


Figure 1: Concentration of free L-carnitine in plasma of pigs at various time points after oral administration of various L-carnitine compounds. Data are means, n = 5 –6 per group

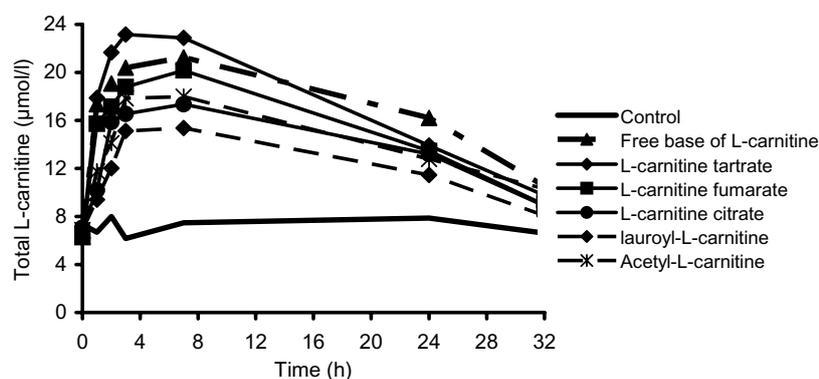


Figure 2: Concentration of total L-carnitine in plasma of pigs at various time points after oral administration of various L-carnitine compounds. Data are means, n= 5 –6 per group

Conclusion

Oral application of L-carnitine leads to an increase in the plasma L-carnitine concentration. Our study, moreover, shows that the time course of the increase of the plasma concentration is similar for free L-carnitine and total L-carnitine. The data of our study suggest that most of the L-carnitine absorbed from different dietary L-carnitine compounds enters the blood as free L-carnitine. This study suggests that L-carnitine salts have a similar bioavailability as base of free L-carnitine while L-carnitine esters have a lower one. The study also suggests that L-carnitine L-tartrate is absorbed fast than the other L-carnitine compounds.

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Effects of L-carnitine supplementation on the somatotrophic axis and masses of placentae in pregnant sows

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Introduction

Recent studies have shown that L-carnitine supplementation in pregnant sows improves intrauterine growth of piglets (Musser et al. 1999, Eder et al. 2001, Ramanau et al. 2004). The biochemical mechanisms underlying this effect are largely unknown. L-carnitine increases plasma concentrations of insulin-like growth factor 1 (IGF-1) (Musser et al. 1999). IGF-1 and IGF-2 are important hormones for placental development and transport of nutrients across the placental barrier (Sterle et al. 1995).

We hypothesize that dietary L-carnitine supplementation in pregnant sows enhances the development of placentae mediated by influencing plasma concentrations of hormones of the somatotrophic axis. To investigate this hypothesis, we determined concentrations of insulin, IGF-1, IGF-2, IGF-binding protein 3 (IGFBP3), somatostatin and somatotropin in sow's blood at 80th day of pregnancy and chorion weights at birth. We determined protein concentrations in chorion as an indicator of protein synthesis capacity and DNA concentrations as an indicator of cell number (Rehfeldt et al. 2001). To achieve information about the capacity of nutrient transfer, we determined protein concentration of glucose transporter 1 (GLUT-1).

Materials and methods

Experiment 1: This experiment involved 40 gilts (German Landrace x Large White) which were allotted to two groups of 20 sows each. Sows of the treatment group received 125 mg L-carnitine per day and head as tablets (Lohmann Animal Health, Cuxhaven, Germany). Sows of the control group received placebo tablets without L-carnitine. After artificially insemination 35 animals conceived (19 in treatment group and 16 in control group). Blood samples were taken at the 80th day of pregnancy. As only 30 single farrowing pens were available, only 15 sows of L-carnitine group and 14 sows of control group (two sows must be excluded, because of osteochondrosis of knee joints) were recorded for their reproductive performance. We determined

concentrations of insulin, IGF-1, IGF-2, IGFBP-3, somatostatin and somatotropin in blood by using of commercial available enzyme-linked-immunosorbent- and radio-immunosorbent assays. Effects were analyzed by Wilcoxon rank sum test and Student's *t*-test (SAS version 9.1.3., SAS Institute, Cary, NC, USA).

Experiment 2: This experiment involved 12 sows in third parity (6 per group). The experimental conditions conformed with those of Experiment 1. In the treatment group 6 sows became pregnant, in the control group 5 sows became pregnant. At birth, all chorions of each sow were collected. We recorded individual weights of all chorions and took tissue samples upside umbilical cord. DNA and protein were isolated with TRIZOL (Invitrogen, Life Technologies, Karlsruhe, Germany), quantified with PicoGreen[®] dsDNA Quantitation Reagent (Molecular Probes, Leiden, Netherlands) and Bicinchoninic acid (PIERCE, Uptima, interchim, Montlucon, France). The concentration of GLUT1 protein was determined by Western blot. Data of chorions were analysed with a mixed linear model (group as fixed effect, sow as random effect) (SAS, version 9.1.3., SAS Institute, Cary, NC, USA).

Results

Experiment 1: Sows supplemented with L-carnitine had higher concentrations of total L-carnitine in plasma than control sows ($11 \pm 4 \mu\text{mol/L}$ vs. $8 \pm 2 \mu\text{mol/L}$, $P < 0.15$). Plasma concentrations of IGF-1 (control sows vs. L-carnitine supplemented sows: $3.9 \pm 2.4 \text{ nmol/L}$ vs. $5.6 \pm 2.2 \text{ nmol/L}$, $P < 0.05$) and IGF-2 ($10 \pm 8 \text{ nmol/L}$ vs. $25 \pm 21 \text{ nmol/L}$, $P < 0.05$) were higher while serum concentration of IGFBP-3 was lower ($0.52 \pm 0.06 \text{ nmol/L}$ vs. $0.60 \pm 0.15 \text{ nmol/L}$, $P < 0.05$) in sows supplemented with L-carnitine than in control sows. Concentrations of insulin in serum (control sows vs. L-carnitine supplemented sows: $88.3 \pm 50.4 \text{ pmol/L}$ vs. $73.0 \pm 34.3 \text{ pmol/L}$, $P = 0.62$), somatotropin in plasma ($2.93 \pm 3.59 \text{ ng/ml}$ vs. $1.47 \pm 0.81 \text{ ng/ml}$, $P = 0.62$) and somatostatin in plasma ($28.5 \pm 30.7 \text{ pmol/L}$ vs. $23.2 \pm 16.2 \text{ pmol/L}$, $P = 0.60$) were not different between both groups of sows.

The total number of piglets born and piglets born alive did not differ between the groups (control sows vs. L-carnitine supplemented sows: total number: 10.9 ± 3.1 vs. 10.2 ± 2.2 , $P = 0.51$; piglets born alive: 10.1 ± 2.8 vs. 10.1 ± 2.2 , $P = 1.0$). The number of stillborn piglet however was lower in sows supplemented with L-carnitine than in control sows (0.1 ± 0.4 vs. 0.8 ± 0.8 , $P < 0.05$). The weight of piglets and litters at birth

was slightly higher (+6%, P=0.45; +9%, P=0.27, respectively) in L-carnitine treated sows than in control sows, but the differences were not statistically significant.

Experiment 2: The number of piglets born (control sows vs. L-carnitine supplemented sows: 14.6 ± 1.8 vs. 12.7 ± 3.2) and piglets born alive (13.4 ± 1.8 vs. 12.5 ± 3.0) did not differ significant between the two groups of sows. Sows with L-carnitine supplementation had a lower number of stillborn piglets and heavier piglets at birth than control sows (0.2 ± 0.4 vs. 1.2 ± 0.8 , P<0.05; 1.62 ± 0.30 vs. 1.44 ± 0.12 , P=0.22).

Sows supplemented with L-carnitine showed a tendency towards heavier chorions than control sows (Table 1). GLUT1 protein concentration in chorion of L-carnitine supplemented sows was higher (62%, P<0.05) than in chorions of control sows.

Table 1: Weights of chorion and amounts of DNA and protein in chorion of control and sows supplemented with L-carnitine^a

parameters	control	+ L-carnitine 125 mg/d
chorion weight [g]	264 ± 51	323 ± 51 ⁺
DNA [mg/chorion]	3.57 ± 1.12	4.92 ± 1.18 ⁺
protein [g/chorion]	11.2 ± 1.90	16.2 ± 2.06 [*]

^a Values are means ± standard deviation (n=5 for control, n=6 for + L-carnitine)

* P<0.05 compared to control group; ⁺ P<0.10 compared to control group

Discussion

Recent studies have shown that dietary L-carnitine supplementation in pregnant sows increases plasma total L-carnitine concentration (Musser et al. 1999, Ramanau et al. 2004). The total L-carnitine concentration in plasma of treatment group was 38% higher than in control group. Although this increase was not statistically significant, it confirms the results of Musser et al. (1999).

The finding that L-carnitine increases plasma IGF-1-concentrations agrees with observations of Musser et al. (1999). Rehfeldt et al. (2004) reported that changes in IGFBP3 plasma concentrations may lead to changes in concentrations of free IGF-1. The increased IGF-1, IGF-2 and decreased IGFBP3 plasma concentrations suggests that IGFs were more available to target tissues in sows supplemented with L-carnitine than in control sows. It has been shown that porcine growth hormone treatment of sows increases maternal plasma IGF-1 concentrations and placental weight (Sterle et al. 1995). L-carnitine treatment in pregnant sows also increased plasma IGF concentrations in Experiment 1 and weights of chorions in Experiment 2. Chorions of

sows supplemented with L-carnitine showed a tendency towards a higher cell number and a higher protein content as chorions of control sows. These data suggest that L-carnitine supplementation in pregnant sows led to a hyperplasia of chorion tissue. GLUT1 protein concentration in basal membrane is rate-limiting for the transport of glucose from maternal to fetal blood. Therefore, it is likely that an increased GLUT1 concentration led to an increased transfer of glucose through placenta (Illsey 2000).

In conclusion, this study shows that L-carnitine supplementation of sows increases plasma concentrations of IGFs in late pregnancy, masses of chorions and GLUT1 protein concentration in chorion. This suggests that L-carnitine enhances placental development and intrauterine nutrition of the fetuses by increased maternal plasma IGF concentrations.

Summary

Two experiments were performed to investigate effects of dietary L-carnitine supplementation on the concentrations of hormones of the somatotrophic axis (IGF-1, IGF-2, IGFBP3, insulin, somatostatin, somatotropin) in pregnant sows and masses of chorions at birth. Experiment 1 shows that L-carnitine decreases number of stillborn piglets at birth, increases plasma concentrations of IGF-1, IGF-2 and decreases plasma concentration of IGFBP3. Experiment 2 shows that L-carnitine increases masses of chorions, amount of protein and DNA and GLUT1 protein concentration in chorion. It is suggested that dietary L-carnitine enhances the development of the placenta and the nutrition of fetuses by an increased maternal IGF-1 concentration and thus might be the reason for increased birth weights of piglets observed recently.

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Impact of fish n-3 polyunsaturated fatty acids on growth of the HT29 human colon tumour cell line.

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Objective: Colorectal cancer is one of the most common cancers in western countries. Epidemiological studies suggest a high fish intake to be associated with a decreased risk for colorectal cancer, but research is still needed to improve understanding of cancer preventive mechanisms of such a diet. Therefore the major fatty acid constituents of fish oil, namely the n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are thought to be the bioactive compounds in fish, were used for investigating growth modulatory effects on HT29 colon tumour cells. Methods: The effect of ethanol dissolved fish PUFAs EPA (C20:5, n-3) and DHA (C22:6, n-3) in comparison to the common plant PUFA linoleic acid (LA, C18:2, n-6) [10-1000 µM] on growth of the HT29 human colon tumour cell line was determined. For this we used the 4',6-Diamidino-2-phenylindole (DAPI) assay for DNA quantification as well as the Cell Titer Blue (CTB) assay for measuring mitochondrial metabolic activity after 24, 48 and 72h treatment. Results: Both fish n-3 PUFAs EPA and DHA reduced the DNA amount and mitochondrial metabolic activity of HT29 colon tumour cells in a dose- and time-dependent relationship. In comparison to EPA DHA showed a stronger inverse association on DNA amount (DAPI EC₅₀ 24h 555 vs. 178 µM; 48h 431 vs. 135 µM; 72h 390 vs. 117 µM) as well as on the mitochondrial metabolic activity (CTB EC₅₀ 24h 926 vs. 291 µM; 48h 764 vs. 185 µM; 72h 651 vs. 155 µM). A treatment with LA had neither an impact on the DNA amount, as a mirror of the cell number, nor on the mitochondrial metabolic activity. High concentrations of all PUFAs (≥ 500 µM) showed microscopically detectable cytotoxic effects. Conclusions: Our data show, that both fish n-3 PUFAs EPA and DHA are potent inhibitors of colon tumour cell growth by affecting cell numbers (calculated from the DNA amount) and metabolic activity of HT29 colon tumour cells in contrast to the n-6 PUFA LA.

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Effect of conjugated linoleic acid on growth and nutrient digestibility in dogs

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Introduction

A large number of conjugated linoleic acid (CLA) isomers occur naturally in ruminant lipids. Two isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12, are known to have biological activity including modulation of fatty acid metabolism and suppression of chemically induced tumours (Fritsche and Steinhart, 1998; Banni et al., 1999, 2003). Inhibition of lipogenesis in adipocytes and down regulation of Δ -9 desaturase activity in a number of tissues is attributable to the *t*10, *c*12 isomer (Park et al., 1999; Pariza et al., 2001). It has been found that CLA affects body composition in various mammals including mice, rats, pigs and humans by increasing the lean muscle mass and decreasing fat, especially depot fat. An improved feed conversion rate was reported when CLA was added to the diets of growing pigs (Dugan et al., 1997, Pfeiffer et al., 2002).

Materials and Methods

Two studies were conducted to investigate the effects of CLA on growth performance and nutrient digestibility in dogs. In both studies, there were two treatments (control: 0.5% sunflower oil, 0% CLA; treatment: 0.5% CLA, 0% sunflower oil). The CLA source (Luta-CLA[®] 60, BASF AG Germany) contained in total 63.5 % of the desired CLA isomers (*c*9, *t*11 and *t*10, *c*12) in a 1:1 ratio (Table 1). The diets were formulated to meet AAFCO standards and to be isoenergetic. The dietary nutrient content is shown in Table 2. In Study 1, twenty adult mixed breed dogs (10 males and 10 females) were allotted to treatments by sex and weight. Dogs were housed individually in kennels that measured 2m x 3m. Dogs were provided exercise 30 min. each day. Feed and water were offered *ad libitum*. Body weight and feed intake were measured weekly during the study duration of 25 weeks.

Table 1: Product composition of Luta-CLA®60

CLA 9c,11t methyl ester:	31.6%
CLA 10t,12c methyl ester:	31.9%
Other isomers (c,c and t,t):	< 1% each
Oleic acid methyl ester:	22%
Palmitic acid methyl ester:	6%
Stearic acid methyl ester:	4%
Linoleic acid methyl ester:	2%
Methanol:	< 100 ppm
Heavy metals:	< 1 ppm

Table 2: Nutrient content of diets

Parameter	CLA	Control
Dry matter, %	91.6	93.0
Fat, %	8.2	4.7
Fiber, %	1.8	2.3
Ash, %	6.4	6.6
Gross energy, MJ/kg	19.97	19.35

In Study 2, six Beagle dogs per treatment were used to compare nutrient digestibility of the experimental diets (0% CLA and 0.5% CLA). Dogs were housed individually in metabolism crates. Total faeces collection was done over a 5-day period.

Results

The diets were not isoenergetic, due to a fault in diet preparation. In Study 1, there was a positive trend ($P>0.05$) for the dogs fed CLA to have lower weight gain (1.05 kg for CLA vs. 2.13 kg for control, after 25 wk). During the entire study the dogs in the CLA group gained 6.2 % of their initial body weight, while dogs in the control group gained 12.4 % (Fig. 1).

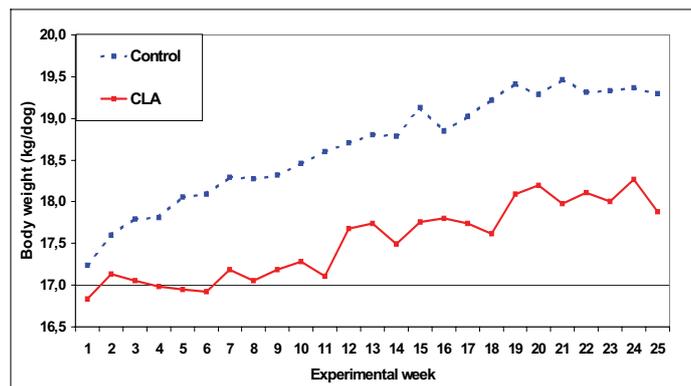


Figure 1: Body Weight of Dogs

However, average daily ME intake was similar between the two treatments (Figure 2).

Thus, the difference in body weight gain was not due to a difference in energy uptake.

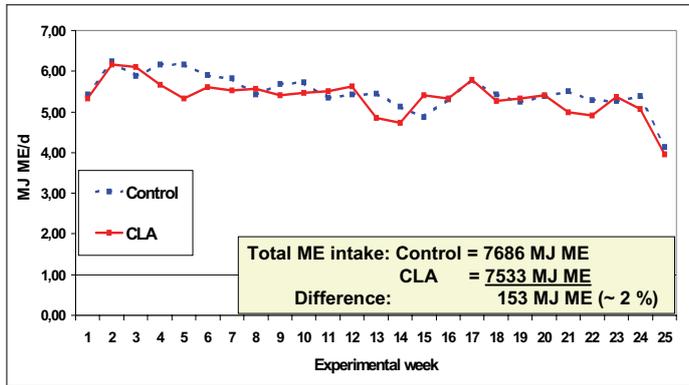


Figure 2: Average daily energy intake of dogs in Study 1.

The results from Study 2 show that the addition of CLA did not have a negative impact on the nutrient digestibility of the diet. No significant differences ($P > 0.05$) in digestibility coefficients between the two treatments were found (Table 3).

Table 3: Nutrient digestibility of experimental diets

Parameter	CLA	Control
Dry matter	85.6 ± 2.9	86.1 ± 2.4
Energy	87.5 ± 2.8	87.2 ± 2.1
Protein	83.8 ± 4.7	85.2 ± 2.7
Fat	95.3 ± 1.0	93.6 ± 1.5
NFE	89.9 ± 2.1	91.4 ± 1.4
ME, MJ/kg DM	16.90 ± 0.59	16.16 ± 0.53

Conclusions

Results from Study 1 indicate that CLA has potential to limit the rate of weight gain in adult dogs. Results from Study 2 demonstrate that dietary CLA at 0.5% of the diet does not affect nutrient digestibility.

Summary

Two studies were conducted to investigate the effects of dietary CLA (0.5%) on growth performance and nutrient digestibility in adult dogs. The CLA source (Luta-CLA[®] 60) contained a mixture of CLA isomers c9, t11 and t10, c12. Study 1: 20 adult mixed breed dogs were allotted to treatments by sex and weight and housed individually. Feed and water were provided *ad libitum*. ME intake was similar in both groups. Over the entire experiment, there was a positive trend for the dogs fed CLA to have lower weight gain (1.05 kg for CLA vs. 2.13 kg for control). Study 2: six beagle dogs/treatment were used to compare nutrient digestibility of the diets. There were no differences ($P>0.05$) in digestibility coefficients between the treatments. Results indicate that CLA has potential to limit the rate of weight gain in adult dogs, while having no negative impact on nutrient digestibility.

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The use of sodium butyrate (adimix c) in layer nutrition

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Introduction

Research in human and animal nutrition has clearly shown that the intestinal epithelial cells utilize butyrate as a primary energy source. Therefore it can be hypothesized that butyric acid enhances nutrient absorption, and hence may lead to improved technical performance in laying hens. Especially in older layers (decreasing laying performance, bone demineralization), improved absorption of calcium and phosphorus may be beneficial. In this study, the effect of the inclusion of butyric acid on lay performance at the end of the lay cycle was evaluated.

Material and methods

A feeding trial consisting of 5 treatments with 7 repetitions of 20 ISABROWN layers was set up with increasing concentrations of Sodium Butyrate (ADMC): 0, 50, 100, 250 and 500 ppm. ADMC is a 98 % sodium-butyrate product (79 % butyrate on total weight) produced and marketed by Nutri-Ad International. ADMC was added to a high quality wheat/corn based feed that was fed over a period of 28 days. Animals were 54 weeks of age at the start of the trial and were housed at a density of 450 cm² / bird on an 18L:6D h light scheme.

The feed formulas are given in Table 1, the feed composition in Table 2.

Eggs from a 24 h lay period were collected biweekly to evaluate the following parameters:

Technical performances

- laying performance,
- average egg weight,
- daily egg mass output
- feed efficiency

Egg bending to evaluate the effect of butyrate on the eggshell strength.

After six weeks, 5 eggs with normal egg bending characteristics (15-30 µm) and with egg weights between 65 and 70 g were taken from each repetition in order to determine the ratio egg yolk, egg albumen and egg shell on total egg weight, and to determine the amount of Ca and P of the egg shell.

All zootechnical parameters were subjected to a 2-factorial analysis of variance "Diet (n=5)*Block (n=7)" and LSD-multiple range test (STATISTICA, 1997).

Table 1: Feed composition (%)

Nr	Name	Contents (%)
300	Corn	20.00
800	Wheat	42.90
1400	Fullfat soybean HT	13.80
1420	Soy 48+2	4.80
1623	Sunflower extract 28 % CP	7.50
5100	Bicalciumphosphate	0.70
5150	Limestone white powder	1.82
5152	Lime white granular	5.50
5170	Salt fine dry	0.23
5173	Sodium bicarbonate	0.15
5301	Methionine	0.10
	Premix Layer 2.5 %	2.50
	Sum	100

Table 2: Theoretical chemical composition of feed (g/kg)

Component	Content (g/kg)
Dry matter	879
Crude ash	112
Crude protein	160
Fat	41.6
Starch	398
Crude fibres	45.4
Ca	31.8
Total P	5.1
Av. P	3.4
Dig Meth + Cyst poultry	5.54
Dig Meth/dig meth+cyst poultry	0.58
Dig lys/Dig Meth + Cyst poultry	1.04
Dig threon/Dig Meth + Cyst poultry	0.81
Dig trypt./Dig Meth + Cyst poultry	0.29
ME _n Layer (kCal)	2750
ME _n layer (MJ)	11.5
ME _n poultry (kCal)	2700
ME _n poultry (MJ)	11.3

Results and Discussion

Results indicate undoubtedly a positive effect of Sodium Butyrate on laying performance. The percentage lay increased from 83.1% (control) to 86.6 % (500 ppm ADMC; Table 3). As average egg weight was not affected, this higher lay performance led to the daily egg mass output increasing from 55.2 g/a/d (control) to 56.4 g/a/d (500 ppm ADMC). Feed conversion was improved by the use of ADMC from 2.111 (control) to 2.070 (500 ppm ADMC) since ADMC feeding did not lead to a higher feed intake. These positive effects were already observed within 2 weeks after the start of the addition period and continued for the full period (Fig. 1). ADMC had an effect on the egg bending as the percentage of eggs having egg bending of more than 30 μm or more decreased from about 8 % to about 3 % from 100 ppm ADMC in the diet.

Table 4 gives an overview on the egg composition (% of shell, yolk, albumen) and Ca and P contents of the eggshell. No effect of ADIMIX C on percentage shell, yolk and albumen could be noticed. The Ca content of the eggshell in the various treatments showed a numerical raise in line with the concentration of ADIMIX C. However the P levels increased substantially in the shell with increasing dosage of ADIMIX C. This is important to avoid chalky deposits in the eggshell and secure eggshell quality.

Table 3: Technical performance data

ADMC (ppm)	Lay efficiency (%)	Average egg Weight (g)	Average egg Mass (g/a/d)	Daily feed Intake (g/a/d)	FCR
0	83.1a	66.4	55.2	116.5	2.111a
50	83.8ab	66.1	55.4	116.1	2.099ab
100	84.3ab	65.9	55.5	116.2	2.094ab
250	84.8ab	66.5	56.4	116.8	2.074b
500	86.6b	65.5	56.4	116.6	2.070b

Values in a column followed by the same letter are significantly not different ($p < 0.05$)

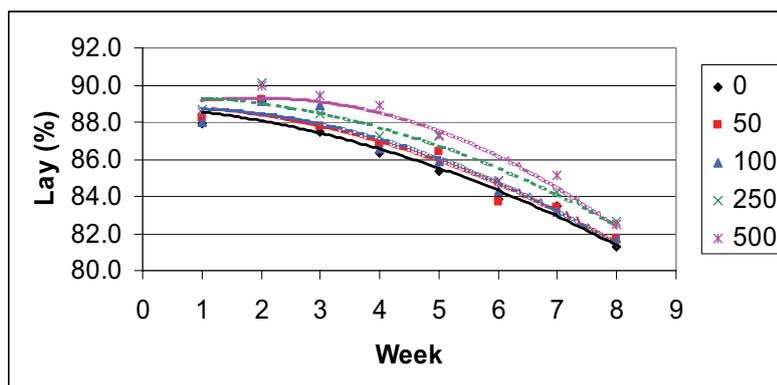


Figure 1: Laying curve based on weekly performance

Table 4: Egg composition parameters

ADMC (ppm)	0		50		100		250		500	
Parameters	Av.	Std								
Shell/total egg (%)	10.6	0.4	10.4	0.3	10.6	0.8	10.4	0.1	10.7	0.3
Yolk/total weight (%)	28.8	0.7	29.0	0.6	28.8	1.3	29.6	1.0	28.2	1.0
Albumin/tot Weight (%)	60.6	1.0	60.6	1.1	60.6	2.0	60.0	1.4	61.1	1.6
Ca (g/kg)	362.4	3.1	365.1	2.5	362.6	2.6	363.4	3.7	364.4	2.8
P (g/kg)	1.09	0.06	1.09	0.10	1.14	0.01	1.16	0.04	1.27	0.02

Conclusions

There is a clear dose response from the diet supplementation of sodium-butyrate (ADIMIX C) in the laying performance of hens in the period after they reached the peak of lay and remaining the egg composition parameters. The best results in percentage lay were achieved at the dose rate of 500 ppm ADMC.

In other trials with layers and breeders an improvement in lay performance (increased % lay, larger egg size) have been noticed when sodium butyrate was supplied two weeks before the hens are in production.

Effect of dietary supplementation with algal omega-3 lipids on fatty acid composition of egg yolks

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Introduction

Changes in the composition of the feed of laying hens can influence composition and nutritional value of eggs. This possibility has been used to produce so-called “functional eggs” with lower cholesterol and saturated fatty acid content, increased iodine or omega-3 fatty acids content. Omega-3 enriched eggs are interesting because they can be valuable sources of eicosapentaenoic and docosahexaenoic acids (EPA and DHA). These two acids have special biological role in human organism and food producers are considering new ways for enriching the food supply with them (1). Different omega-3 sources are used for dietary supplementation of hens, as flaxseed and fish meal. In EU, Australia and some other countries the material from marine algae *Schizochytrium* (microalgae itself or DHA-rich oil) has been approved for human consumption as a novel food ingredient (2,3) and is also being used as a feed ingredient. The aim of this experiment was to investigate the effect of hen’s diet supplementation with DHA-rich marine algae *Schizochytrium* on fatty acid composition of egg lipids.

Materials and Methods

Sixty 30-weeks old crossbred Lohman LSL-Classic laying hens were randomly divided into three experimental groups: Control – basal diet based on corn and sunflower oil with added vitamin-mineral mixture; Group 1 – basal diet + 0.7% DHA-rich marine algae supplement; Group 2 – basal diet + 1% DHA-rich marine algae supplement. Feed and water were provided *ad libitum*. The feeding regimen lasted one month. Fatty acids of egg yolk lipids were analyzed at the beginning of the experiment, after 7 and 30 days.

Fatty acid composition of lipids extracted according to Folsch procedure was estimated using gas chromatography/FID detector on packed column (Varian Chromatograph model 1400; fatty acid methyl esters standard Supelco™ FAME Mix).

The significance of differences between experimental groups was tested using Student T test.

Results and Discussion

Analysis of fatty acid composition of marine algae *Schizochytrium* feed additive showed that more than 40% of all fatty acids was omega-3 DHA, but also that docosapentaenoic acid (DPA, omega-6) was present with 18% (Table 1).

The most pronounced effect of feeding marine algae to laying hens was significant increase in DHA in both supplemented groups ($p < 0.01$). Adding 1% marine algae to basal feed led to significantly higher levels of DHA in comparison with 0.7% ($p < 0.01$). Other authors also reported that increasing levels of dietary polyunsaturated fatty acids (PUFAs) result in increase of their concentration in egg yolk (4). The DHA levels in yolk lipids were higher after period of 30 days in comparison with the period of 7 days, but this difference was significant only in group 1 (Figure 1).

Table 1: Fatty acid composition of microalga

Schizochytrium feed additive

Fatty acid	%	Fatty acid	%
12:0	0.7	18:2, n-6	0.2
14:0	7.0	20:4, n-6	3.5
15:0	0.9	20:4, n-3	1.1
16:0	20.3	20:5, n-3	3.8
18:0	1.0	22:5, n-6	18.1
18:1	0.2	22:6, n-3	41.4

Although the level of DPA in feed supplement was high, this fatty acid was not incorporated into the yolk lipids. This fatty acid was probably used as a source of energy for hens in beta-oxidation process. The amount of arachidonic acid (20:4, omega-6) in egg yolk lipids decreased in supplemented groups which is in accordance with the findings of Schreiner et al (5).

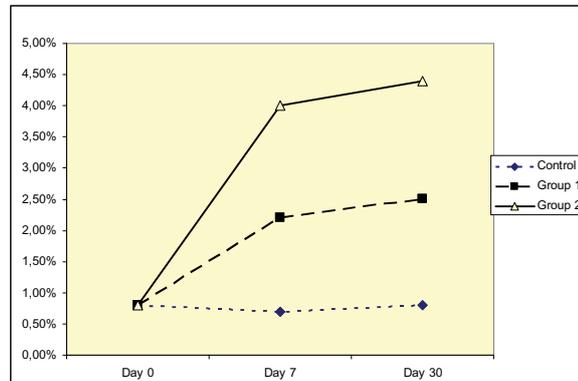


Figure 1: DHA content in yolk lipids (%)

In the study of Herber et al (6) other marine algal feed additive also promoted DHA deposition in egg yolk lipids, indicating that marine algae could be valuable sources of selected omega-3 fatty acids and an alternative to other currently used omega-3 dietary sources in animal feeding process.

DHA and total PUFA contents in control eggs and eggs from hens supplemented with 1% of algal additive are presented in table 2. The level of DHA increased 6 times, while total PUFA content increased 9%.

Table 2: Fatty acid content of egg yolks (mg/100 g whole egg, without shell)

Fatty acids	Control	Group 2/Day 30
Polyunsaturated fatty acids (PUFAs)	1130	1220
Docosahexaenoic acid (DHA)	22	130

The health benefits of dietary omega-3 fatty acids are well known, but also are known that usual western diet is deficient in these biologically active compounds (7). The consumption of fish (common dietary source of EPA and DHA) is usually low, therefore the development and production of foods enriched with omega-3 fatty acids such as eggs or other foods of animal origin could be efficient way in increasing their dietary intake to recommended levels.

Summary

This experiment was conducted to evaluate the effect of marine marine algae *Schizochytrium* added to usual feed of laying hens on distribution of polyunsaturated fatty acids in egg yolk lipids. Sixty 30-weeks old crossbred Lohman LSL-Classic laying hens were randomly divided into three experimental groups: control – basal diet based on corn and sunflower oil; Group 1 – basal diet + 0.7% DHA-rich marine algae supplement; Group 2 – basal diet + 1% DHA-rich marine algae supplement. The feeding regimen lasted one month. Fatty acids of egg yolk lipids were analyzed at the beginning of the experiment, after 7 and 30 days. Feeding marine algae supplement at both levels led to significant increase of docosahexaenoic acid in egg yolk lipids ($p < 0.01$) and concomitant decrease in arachidonic acid which was not significant. Although the marine algae supplement had a high amount of docosapentaenoic acid (DPA), its level did not change throughout the experiment.

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Adipose tissue lipids of pigs fed sunflower oil supplemented diet

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Introduction

The addition of nutritive fats to the ration of pigs has a favourable effect on their growth and development by importing some essential components and improving the caloric density of the diets. Lipid metabolism and the development of their adipose tissue, are intensively studied because due to its propensity to synthesize and deposit large amounts of fat (Diersen-Shade et al., 1988; Nguyen et al., 2003). Pig adipocytes accumulate triacylglycerols by the esterification of glycerol-3-phosphate with fatty acids. Glycerol-3-phosphate is synthesized in adipocytes from glucose, while fatty acids can be synthesized de novo in the adipocytes or be obtained from plasma lipoproteins. However, the exact mechanism by which nutritive lipids affect lipogenic processes has not been completely clarified yet. The object of the study is to follow the effect of the added sunflower oil to the mixture for test pigs on the activity of lipogenic enzymes, the composition and content of main lipid classes in adipose tissue – fresh and after prolonged storage in vacuum packages at -25°C .

Materials and methods

The experiment is carried out with 12 male castrated pigs (Large White x Duroc), bred in individual boxes, from 20 to 60 kg live weight. The control pigs are fed with standard corn-soybean mixtures, while to the mixture of the test pigs is added 2% sunflower oil. The rations are isoproteinic and isoenergetic. Adipose tissue samples are taken from subcutaneous adipose tissue at the last cervical vertebra immediately after slaughtering and are divided into three equal parts: for immediate analyse (Ist period), vacuum packed and stored for three months (IInd period) and six months (IIIrd period) at -25°C . The total lipids are extracted according to Bligh and Dyer (1959). The content and the fatty acid composition of triacylglycerols (Tg) and free fatty acids (FFA) are determined by a combined application of thin-layer and gas chromatography (Cunnane et al., 1986). The fatty acid methyl esters are separated by a gas chromatograph equipped with a capillary column. The total cholesterol is

evaluated by the procedure of Sperry and Webb (1950). Malic enzyme (EC 1.1.1.40; ME) is assessed by the method of Ochoa (1955), and glucose-6-phosphate dehydrogenase (EC1.1.1.49; G-6-PDH)- by the method of Glock and McLean (1953).

Results

The adipose tissue of the experimental animals, compared to that of the control ones showed lower activity of NADP-malate dehydrogenase ($P \leq 0.001$; Tab.1), an enzyme that delivers reducing equivalents for the synthesis of fatty acids de novo. This may be due to the inhibiting effect of sunflower oil added to the test group's mixture (Klimes et al., 1993; Sebkova et al., 1994). Regardless this, the higher Tg level in the test pigs is probably due to their direct import through feed. The total FFA concentration showed a moderate level of lipolyse in adipose tissue in vivo (Ist period). The storage of three and six months is accompanied by a considerable increase in lipolytic processes leading to an increase in the pool of FFA ($P \leq 0.001$) in the two groups of animals. In test animals the cholesterol values in fresh tissue show a tendency for some decrease, which is similar to the cholesterol-lowering effect of nutritive oils. It can be noticed that the obtained values are much lower – under 100 mg % compared to those established by Harris et al., (2003) in experiments with nutritive oils added to pigs diets. Probably this is a specific character of the tested hybrid and can be related to the cholesterol-lowering effect of the soybean protein in the rations (Lovati et al., 1992).

Table 1. Content of main lipid classes and activity of lipogenetic enzymes in adipose tissue from pigs fed sunflower oil supplemented diet

Groups	Parameters	Triacylglycerols, $\mu\text{M}/100\text{g}$	Free fatty acids, $\mu\text{M}/100\text{g}$	Cholesterol, $\text{mg}/100\text{g}$	Malic enzyme	G-6PDH
Control	I period	95072±708	1500±167	96±5	75.71±4.05	50.27±4.83
	II period	91088±554	1638 ^a ±714	96±1	-	-
	III period	90462±2464	2234 ^b ±138	107±3	-	-
Experimental	I period	96232±719	1197±104	89±3	36.50±2.1*	61.92±2.61**
	II period	89743±1498	1745±201	93±2	-	-
	III period	91109 ^c ±1078	2001 ^c ±164	101 ^c ±3	-	-

Enzyme activity expressed as nM/ min/ mg protein at 25°C; *P <0.05; **P <0.001

a- control group I period/ control group II period;

b- control group I period/ control group III period;

c- experimental group I period/ control group II period;

d- experimental group I period/ control group III period

The fatty acid composition of adipose tissue Tg in test pigs (Fig. 1) shows a decrease in the level of 16:0 and increase in the molar concentration of the essential 18:2

($P \leq 0.05$). These changes, as well as the tendency to increase in the content of 18:3 remind of the influence of alimentary oil.

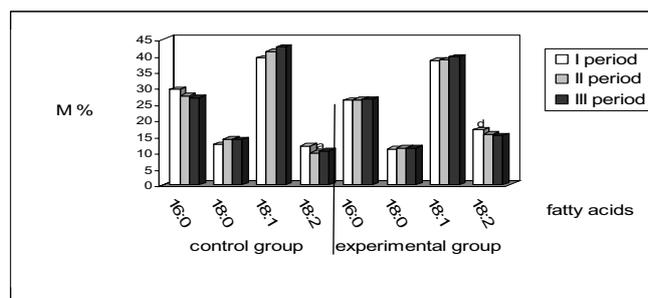


Figure 1. Fatty acid composition (M %) of adipose tissue triacylglycerols from pigs fed sunflower oil supplemented diet

However, the established changes are considerably weaker than those reported by other authors in cases of unsaturated oils addition to the diets of test animals (West and Myer, 1987; Nguyen et al., 2003), especially regarding 18:2. This could be related to the lower level of the added oil and to the specificity of the hybrid. The enrichment of the adipose tissue Tg of test animals with 18:2 and 18:3, is favourable to the taste characteristics and nutritional value of the obtained product. The resulting ratio of fatty acids does not increase unfavourably the total unsaturation, related to the deterioration of back fat texture and the possibility for its technological processing. After storage, mainly after six months, in the Tg of control pigs a quantitative decrease to 16:0 and 18:2 ($P \leq 0.05$) is established - a possible and emphasized change. The Tg of the experimental animals show changes in the same direction as in the control group, but are less expressed, and the concentration of 18:2 remains definitively higher than that of control pigs till the end of the tested period. The fatty acid profile of FFA (Fig. 2) shows a higher level of 16:1, 18:1 and 18:2 ($P \leq 0.05$) and lower concentration of 16:0 ($P \leq 0.05$) in test animals compared to the control group (1st period) which can be accepted as a reflection of the nutritional factor. The storage, mainly of six months, is accompanied by an increase in the lipolytic processes and changes in the FFA spectrum leading to fatty acid ratios close to those of adipose tissue Tg. This is expressed in lowering of the molar percentage of 16:0, 16:1 and 18:0, simultaneously with an increase of that of 18:1, 18:2 and 20:1 in the control animals. In the test animals, the intensity of lipolyse is quite similar to that in the control group during storage. Regardless the higher degree of

unsaturation, the received values do not reach critical levels, which could lead to the formation of a high oxidation potential, deteriorating the nutritional and technological qualities of the adipose tissue in both experimental and control pigs.

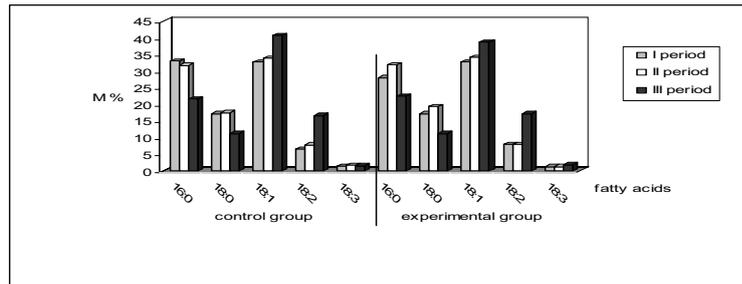


Figure 2. Fatty acid composition (M %) of adipose tissue free fatty acids from pigs fed sunflower oil supplemented diet

Summary

The addition of 2% sunflower oil to the diet of pigs decreases the endogene fatty acid synthesis and affects (in certain limits) in the desired direction the fatty acid composition of adipose tissue lipids, and triacylglycerols are enriched with linoleic acid. Such changes can be considered favourable to the animal and to the consumer, and they do not deteriorate the possibilities for technological processing and storage of the products. The above said gives grounds for a positive evaluation of the opportunities for enrichment of the pigs mixtures for with low quantities of unsaturated oils.

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Influence of long-term intervention with supplemented dairy products on serum lipids

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Introduction

Data from literature show that regular consumption of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) of marine origin can improve serum lipids and reduce cardiovascular risk [Kris-Etherton et al. 2002; Holub & Holub, 2004]. N-3 LC-PUFA are cardioprotective nutrients, because they are precursors for eicosanoids which improve flow properties of the blood, prevent platelet aggregation, have vasodilatory and anti-inflammatory qualities [Calder & Grimble, 2002; Simopoulos 2002].

Long-chain n-3 PUFA modulate blood lipids by lowering VLDL cholesterol and by increasing HDL cholesterol. In addition, n-3 fatty acids lower triacylglycerides which are important risk factors of arteriosclerosis [Singer & Wirth, 2003; Holub & Holub, 2004].

Conventional dairy products are poor in LC-PUFA, but these foodstuffs are rich sources for conjugated linoleic acids (CLA) and short-chain fatty acids. The contents of oleic acid (C18:1, n-9) are beneficial in dairy products and also there are marginal concentrations of arachidonic acid (C20:4, n-6). In this study, dairy products were supplemented by n-3 PUFA.

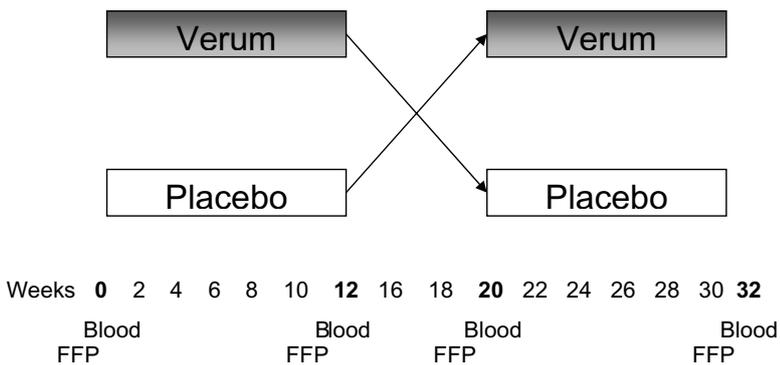
Dairy products like yogurt and cheese were enriched with special oils which are rich in n-3 fatty acids, especially eicosapentaenoic acid (C20:5, n-3, EPA), docosahexaenoic acid (C22:6, n-6, DHA) and alpha linolenic acid (C18:3, n-3, ALA).

This study aimed to analyse the influence of n-3 PUFA supplemented products on serum lipids in humans.

Material and methods

Forty five subjects (43 women, 2 men) were randomised into two groups in a double-blind, placebo-controlled cross-over study. Both groups received placebo products or supplemented dairy products consecutively for three months with a two-month washout phase between both periods (Fig. 1). The daily dose of n-3 fatty acids amounted to 2.3 g, consisting of 1.35 g EPA+DHA and 0.95 g ALA. Clinical examinations were elevated at the beginning and at the end of each period. Each patient had to fill in a food frequency protocol of five representative days. This

protocol assessed the intake of energy, macronutrients, cholesterol, saturated fatty acids, monounsaturated fatty acids and PUFA. The evaluation of the data was carried out with PRODI (version 11.5).



FF: Food Frequency Protocoll (5 days)

Fig.1: Study design

Results and discussion

Thirty eight test persons aged 57.9 ± 10.8 years completed the study. Total cholesterol, LDL cholesterol, LDL/HDL ratio and triacylglyceride were not affected by the intervention with dairy products (Tab. 1,2). Interestingly, HDL cholesterol increased significantly ($p \leq 0.05$) by the consumption of dairy products rich in n-3 fatty acids but increased in the placebo period as well ($p \leq 0.01$; Tab. 1,2). Total cholesterol/HDL cholesterol ratio tended to decrease due to the intake of n-3 fatty acids ($p \leq 0.1$) and decreased significantly in the placebo period ($p \leq 0.01$; Tab. 1,2). Supplementation with dairy products rich in n-3 fatty acids were associated with lower concentrations of lipoprotein(a) ($p \leq 0.05$; Tab. 1,2). This effect was tendencial in placebo period ($p \leq 0.1$).

Table 1: Serum lipids and lipoproteins in *Placebo* intervention
(Mean \pm standard deviation)

	Start value	End value
Total cholesterol [mmol/L]	5,87 \pm 0,94	5,85 \pm 0,88
HDL cholesterol [mmol/L]	1,62 \pm 0,43	1,74 \pm 0,44 **
Total cholesterol/ HDL cholesterol	3,82 \pm 1,06	3,53 \pm 0,88 **
LDL cholesterol [mmol/L]	3,73 \pm 0,85	3,89 \pm 0,92
LDL/HDL	2,44 \pm 0,77	2,44 \pm 0,83
Triacylglyceride [mmol/L]	1,21 \pm 0,53	1,28 \pm 0,99
Lipoprotein(a) [mg/L]	387,33 \pm 436,80	360,66 \pm 408,93 ^t

* Significantly different compared to the start value ($p \leq 0.05$)

** Significantly different compared to the start value ($p \leq 0.01$)

^t Trend towards compared with start value ($p \leq 0.1$)

Table 2: Serum lipids and lipoproteins in *Verum* intervention
(Mean \pm standard deviation)

	Start value	End value
Total cholesterol [mmol/L]	5,79 \pm 0,96	5,87 \pm 0,88
HDL cholesterol [mmol/L]	1,63 \pm 0,37	1,73 \pm 0,39 *
Total cholesterol/ HDL cholesterol	3,67 \pm 0,86	3,52 \pm 0,76 ^t
LDL cholesterol [mmol/L]	3,78 \pm 0,92	3,86 \pm 0,89
LDL/HDL	2,42 \pm 0,81	2,35 \pm 0,78
Triacylglyceride [mmol/L]	1,09 \pm 0,52	1,11 \pm 0,46
Lipoprotein(a) [mg/L]	424,59 \pm 365,10	365,10 \pm 428,04 *

* Significantly different compared to the start value ($p \leq 0.05$)

** Significantly different compared to the start value ($p \leq 0.01$)

^t Trend towards compared with start value ($p \leq 0.1$)

These results show that dairy products modulate serum lipids by increasing HDL cholesterol and lowering lipoprotein(a). The influence of n-3 PUFA was marginal. Possibly, the daily doses of n-3 PUFA supplemented dairy products were too small. The ISSFAL (International Society for the Study of Fatty Acids and Lipids) recommends a daily supply of 0.65 g EPA+DHA. According to the reference values

of Nutritional Societies in Germany, Austria and Switzerland, 0.5% of the daily energy should be taken in form of n-3 fatty acids (1.0 - 1.5 g/d) [D-A-C-H, 2000]. In the literature, preventative effects of n-3 PUFA in doses up to 3 - 4 g/d were described [Singer & Wirth, 2003]. Further studies with higher contents of n-3 LC-PUFA in dairy products are needed to show the cardioprotective effects of these innovative dairy products.

Conclusions

The results show that dairy products influence serum lipids and affect risk factors of cardiovascular diseases, but the supplementation with n-3 PUFA has only marginal influence on serum lipids.

Summary

We conducted a placebo-controlled, double-blind cross-over study with n-3 PUFA supplemented dairy products in 45 volunteers. After 12 weeks of supplementation serum HDL cholesterol was significantly increased in *placebo* period ($p \leq 0.01$) and in *verum* period ($p \leq 0.05$). Total cholesterol/HDL cholesterol ratio tended to decrease due to the intake of n-3 fatty acids ($p \leq 0.1$) and decreased significantly in the *placebo* period ($p \leq 0.01$). Supplementation with dairy products rich in n-3 fatty acids were associated with lower concentrations of lipoprotein(a) ($p \leq 0.05$). This effect was tendencial in *placebo* period ($p \leq 0.1$).

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Assessment of genetically modified prebiotic potato tubers concerning the nutritive value and the fate of DNA

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Introduction

The ability to synthesise high molecular weight fructan as inulin was transferred to potato plants via constitutive expression of the 1-SST (sucrose:sucrose1-fructosyl-transferase) and the 1-FFT (fructan:fructan1-fructosyltransferase) genes of globe artichoke *Cynara scolymus* (Heyer et al., 1999). The fructan pattern of the tubers represents the full inulin spectrum of artichoke roots and the inulin concentration in the dry matter amounts to five percent (Hellwege, 2000). The objective of this study was to analyse the extent to which this modification influences the content of nutrients, their availability to pigs as a model for monogastrics, the content of undesirable substances and the fate of recombinant DNA.

Material and Methods

The transgenic potato lines and the non-transformed line (i.e. cv. *Desirée*) were grown under the same field conditions at an experimental station in 2003. The tubers were steamed and ensilaged. The official VDLUFA-methods were applied to analyse the chemical composition. The nutritive value was determined in balance experiments following the GfE-guidelines. The incorporation rate of the isogenic-or transgenic potato silage in the diet was 40 % DM. The same rations were tested in a subsequent feeding trial with 4 pigs per treatment (49 to 78 kg live weight).

After slaughter digesta samples (stomach, duodenum, jejunum, ileum, caecum, colon and rectum) were taken as well as tissue samples from m. long. dorsi., m. gluteus, thymus, spleen, liver, kidney and kidney fat. For DNA preparation the "Nucleo Spin Food kit" (Macherey-Nagel, Germany) was used. For tracing the DNA derived from the transgenic potato four different PCR-systems were selected.

For all tissue samples a PCR system developed by Laube et al. (2003) was chosen to determine the integrity and quality of the extracted DNA. To identify any inhibition in DNA extracts from the gastro-intestinal tract a sequence was selected from the chloroplast genome. As a single-copy target sequence of the potato genome the metallo-carboxypeptidase inhibitor gene DNA sequence was chosen. To identify the genetic modification in the gastrointestinal tract, a real time PCR system has been developed to target the junction between the Cauliflower mosaic virus (CaMV) 35S promoter and the adjacent *1-ssf* gene within the genetic construct.

Results

The content of nutrients, minerals and amino acids analysed for the silage of both potato variants is summarized in Table 1.

Table 1. Chemical composition of potato silage

	Isogenic	Transgenic
DM (%)	21.2	19.5
	Proximates (% of DM)	
OM	94.5	94.2
CP	10.7	10.6
EE	0.6	0.6
CF	2.5	2.8
NfE	80.7	80.2
Starch	67.4	59.9
	Macro-elements (g/kg DM)	
Ca	0.51	0.56
P	2.10	2.20
K	19.60	20.00
Na	0.30	0.73
Mg	0.90	0.84
	Amino acids (g/100 g crude protein)	
Lysine	4.31	4.00
Methionine	1.18	1.39
Cystine	0.95	0.87
Threonine	2.49	2.53

Proximate and mineral contents do not show significant differences. However, the starch content decreased, which is accordance with the result from fresh tubers (13.4vs.15.0%), indicating that the inulin synthesis is limited and does not increase the storage capacity of carbohydrates (Hellwege et al., 2000). The amino acid

profile did also not show meaningful differences in the levels of any of the 16 amino acids measured. However, the alkaloid content of the transgenic tubers was found to be nearly 25% higher than that of the isogenic potatoes.

Table 2. Glycoalkaloid content of isogenic and transgenic potato tubers (mg/kg DM)

	α -Chaconine	α -Solanine	Total alkaloids
Isogenic	524	204	728
Transgenic	652	252	904

The results on apparent digestibility and energetic feeding value as summarized in Table 3 demonstrate no meaningful differences, but the energetic feeding value, which was calculated based on digestible nutrients showed a tendency towards lower values, which was supported by the results of the feeding test (Table 4).

Table 3. Digestibility and energetic feeding value of inulin synthesising potatoes as compared to those of the non-transgenic controls (means \pm SD)

	Digestibility of nutrients (%)		
	Isogenic	Transgenic	P
Organic matter	93.9 \pm 1.5	93.2 \pm 1.2	0.54
Crude protein	76.9 \pm 8.3	73.0 \pm 10.5	0.60
Ether extract	66.3 \pm 16.5	49.9 \pm 19.3	0.33
Crude fibre	81.0 \pm 6.1	72.6 \pm 11.8	0.27
N-free extracts	90.7 \pm 7.1	94.2 \pm 4.3	0.49
MJ ME/kg DM	14.60 \pm 0.90	14.34 \pm 0.21	0.66

Table 4. Production efficiency of isogenic and transgenic potato silage

	Isogenic	Transgenic
ME-intake (MJ/d)	23.19	22.89
Live weight gain (g/d)	711	668
Energy conversion ratio (MJ ME/kg)	32.62	33.73

None of the 110 tissue samples from pigs fed isogenic or transgenic potatoes gave a positive result in PCR with the metallo carboxypeptidase inhibitor gene real time PCR system or the system specific for the genetic modification in the transgenic potato. The limit of detection (LOD) of all real time PCR systems used was determined to be at least 10 genome copies corresponding to approximately 25 pg DNA. With all applied control systems no specific genetic alteration could be detected in any sample except from the stomach content (Table 5).

Table 5. PCR results from pigs fed transgenic potato silage

	MY	MCP	CP	Sst-1		MY	MCP	CP	Sst-1
<u>Digesta</u>					<u>Organic tissue</u>				
Stomach	+	+	+	+	M. long. dorsi	+	n.d.	n.d.	n.d.
Duodenum	+	+	+	n.d.	M. Glutaeus	+	n.d.	n.d.	n.d.
Jejunum	+	+/-	+/-	n.d.	Thymus	+	n.d.	n.d.	n.d.
Ileum	+	n.d.	n.d.	n.d.	Spleen	+	n.d.	n.d.	n.d.
Caecum	+	n.d.	n.d.	n.d.	Liver	+	n.d.	n.d.	n.d.
Colon	+	n.d.	+	n.d.	Kidney	+	n.d.	n.d.	n.d.
Rectum	+	n.d.	+	n.d.	Kidney fat	+	n.d.	n.d.	n.d.

MY = myostatin gene; MCP = metallo-carboxy-peptidase inhibitor gene; CP = chloroplast-specific sequence (multi-copy); Sst-1 = 35s-promotor – sucrose:sucrose 1-fructosyltransferase gene

Summary

To evaluate silage from transgenic inulin synthesising potatoes as compared to that from the parental cultivar, nutrients, undesirable substances, digestibility and production potential were investigated. The chemical composition showed a decrease of starch (67% vs. 60% in the DM), and an increase of the total alkaloids (728 vs. 904 mg/kg DM). The energetic feeding value was calculated to be 14.6 or 14.3 MJ ME/kg DM for the silage of isogenic or transgenic potatoes. Samples of digesta and tissues were analysed with four real time PCR systems. No plant specific DNA or DNA specific for the genome alteration in the transgenic potato were detected in any organ. In contrast, chloroplast specific DNA was detected in the digesta of duodenum, jejunum, colon and rectum. No evidence for the integration of the foreign DNA into the host genome was observed.

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Effect of dietary transgenic maize on absorption and retention of mineral elements

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With the increasing population and the decreasing area of land available for food production the development and use of genetically modified crops is considered as an important tool to ensure global food security. The Global Review of Commercialised Transgenic Crops (James 1999) states that between 1996 and 1999 the area of GM crops grown globally increased from 2 to 40 million ha at adoption rates which are unprecedented and the highest for any new technology in agriculture. While in 2000, the area cultivated had increased, the rate of increase has slowed down due to the controversial nature of the technology. While North America and Argentina were responsible for the vast majority of the area grown, in the European Union growing transgenic feed crops on large areas is limited. In spite of these facts nowadays 60 to 70 % of foodstuffs come into contact with gene technologies (Phipps and Beever, 2000). While the so-called red gene techniques used in medicine were accepted, discussions on the so-called green gene techniques used in agriculture and the food industries are controversial and emotional (Hodges 1999, 2000). Two crops (soya and maize/corn) account for 82% of the GMO acreage. Together with cotton and rapeseed/canola, they account for over 99% of the GMO acreage. One trait, herbicide tolerance, has consistently been the dominant trait during the six-year period 1996-2001, and accounts for 77% of the GMO acreage. Other traits are insect resistance (15%) and stacked genes for both herbicide tolerance and insect resistance (8%) (Greenpeace 2004).

Objective of this study was to determine the effect of conventional and transgenic maize, so-called Roundup Ready (with an introduced gene of glyphosate resistance), on mineral absorption and retention.

Material and methods

The experiment was performed on 30 Wistar rats (75g), divided into three feeding groups. Tests were carried out on conventional (*Zea Mays* L.) - control and two genetically modified Roundup Ready maize grain (RR maize) of the same line (MON-SANTO, USA): RR1 – bought in 2001 and RR2 – bought in 2000. The transgene of glyphosate (N-phosphonomethyl)glycine, produced under the technical name

Roundup, inhibits the activity of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme (Cox, 1998). This transgene encodes an analogous enzyme by origin in *Agrobacterium tumefaciens* sp. Strain, which is not blocked by glyphosate. The tested maize lines were the only dietary source of nitrogen in the diets so that crude protein contained in the maize lines presented 10% of the dietary dry matter.

Composition of experimental diets (% in dry matter):

Tested maize	10 % crude protein (1.6 g N x 6.25)
Oil	8 %
Crude fibre	4 %
Mineral mixture ¹	5 %
Vitamine mixture ²	1 %
Wheat starch	to 100 %

¹ 15 g CaCO₃, 14 g Ca₃(PO₄)₂, 10 g K₂HPO₄, 8 g NaCl, 7 g Na₂HPO₄, 5 g MgSO₄·7H₂O, 0.48 g Fe citrate, 0.45 g MnSO₄·4H₂O, 0.0195 g CuSO₄·5H₂O, 0.0005 g KJ, 0.01 g NaF, 0.04 g ZnCO₃, starch ad 100 g.

² 5000 IU A, 500 IU D₃, 50 mg E, 1 mg K₃, 20 mg C, 20 mg B₁, 20 mg B₂, 10 mg B₆, 50 mg Ca pantotenate, 50 mg nicotinic acid, 1000 mg choline.HCl, 2 mg folic acid, 100 mg inositol, 200 mg p-aminobenzoic acid, 30 mg B₁₂, starch ad 20 g.

The content of nitrogen in the diets with transgenic and conventional maize ranged from 1.39 and 1.42 g N in 100 g original matter. After 8 days of preliminary period, a balance trial was performed from 8th to 14th day of the experiment. Urine and feces were quantifiable collected once per day. Urine was collected over a funnel into flask containing 5 % v/v sulfuric acid. The samples were kept in fridge. Daily faeces samples were frozen at -20° C. Afterwards all collected amount of faeces and urine was homogenized and analyzed (AOAC, 1975).

After dry mineralization in 450°C, cations (Ca, Mg, Na, K, Fe, Zn and Cu) were determined on the flame atomic absorption spectrometer Unicam 939 (Cambridge, UK) and phosphorus by spectrophotometry using a molybdate agent on a Spekol 11 (Carl Zeiss, Jena, Germany).

Results and discussion

Mineral composition of transgenic and isogenic (conventional) maize lines did not differ much, except zinc content (tab. 1). Low content of bone creating mineral elements, mainly Ca, is their characteristic default.

Conclusions

The results of this study, and the previous ones (Chrenkova et al 2002), as well as the fact that plant mineral composition can change dramatically independently on variety and species (Gralak et al 1996a), allow us to conclude that genetically modified (RR) maize revealed equivalence in mineral composition with isogenic maize.

The differences in mineral absorption and retention in rats offered transgenic maize of different origin (year and place of growing) were in many cases bigger than compared to animals fed isogenic maize.

Table 1. Mineral composition of corn dent yellow (NRC 1982), isogenic and transgenic maize grains used in this study (DM basis).

Mineral element	Corn dent yellow	Isogenic Maize	Transgenic Maize RR1	Transgenic Maize RR2
Ca (g/kg)	0.30	0.15	0.15	0.30
P (g/kg)	2.9	1.4	1.8	1.7
Mg (g/kg)	1.4	1.0	0.9	1.1
Na (g/kg)	0.3	0.01	0.02	0.03
K (g/kg)	3.7	3.3	3.0	4.6
Fe (mg/kg)	30.0	58.03	54.11	63.66
Zn (mg/kg)	14.0	34.16	64.26	63.03
Mn (mg/kg)	5.0	14.39	16.04	12.38
Cu (mg/kg)	4.0	4.67	4.91	4.23

Calcium, magnesium, potassium and iron intake, apparent absorption and retention did not differ between groups (tab. 2). It should be mentioned that some negative values of iron retention and absorption were stated. This fact was also observed in the previous study (Gralak et al 1996b). It was suggested that retention and absorption of mineral elements depended on the properties of dietary fibre and changed with prolongation of the experiment. Phosphorus intake was higher in groups offered diets containing genetically modified maize, but significantly higher retention and absorption was stated only in group RR2. Sodium intake was higher in both groups offered transgenic maize, significantly in group RR1, but apparent absorption was equal in reality in all groups (97.0 – 97.5%). However sodium retention was two fold higher ($p \leq 0.05$) in the group RR2 than in the control group (54 ± 5 vs. 25 ± 8 mg/day/rat). The difference in Na retention among groups was caused by lower sodium excretion in urine in rats offered transgenic maize RR2. The Zn intake per experiment and apparent absorption were significantly lower (2.87 ± 0.13 mg) in RR2 group than in other groups (3.40–3.42mg). However Zn retention in RR2 and control groups was similar, and in both significantly lower than in RR1 group. Negative retention and absorption of zinc were probably caused by endogenous secretion of this element into gastrointestinal tract via pancreatic juice (Gralak et al 1999) and other fluids (Oberleas 1996). Although similar Cu intake in all groups and the significantly lower apparent Cu absorption in RR1 group comparing with the others ($4.2 \pm 7.1\%$ vs. 24.6 – 27.7%) were observed, the Cu retention did not differ among groups (-0.040 – 0.067 mg) what probably resulted from regulatory function of kidneys in Cu metabolism.

Table 2. Mineral balance (x \pm SEM).

Mineral element		Conventional Maize	Transgenic Maize RR1	Transgenic Maize RR2
Ca	Intake (g/7d/rat)	0.518 \pm 0.016	0.517 \pm 0.012	0.490 \pm 0.014
	Excreted (g/7d/rat)	0.276 \pm 0.012	0.296 \pm 0.011	0.263 \pm 0.013
	Retention (g/7d/rat)	0.244 \pm 0.012	0.221 \pm 0.011	0.227 \pm 0.009
	Apparent absorption (%)	49.4 \pm 1.7	45.6 \pm 1.7	49.3 \pm 1.8
P	Intake (g/7d/rat)	0.323 ^a \pm 0.010	0.359 ^b \pm 0.008	0.368 ^b \pm 0.011
	Excreted (g/7d/rat)	0.318 \pm 0.014	0.340 \pm 0.014	0.306 \pm 0.014
	Retention (g/7d/rat)	0.005 ^a \pm 0.012	0.011 ^a \pm 0.014	0.062 ^b \pm 0.009
	Apparent absorption (%)	20.7 ^a \pm 2.6	22.8 ^a \pm 2.2	35.8 ^b \pm 2.1
Mg	Intake (g/7d/rat)	0.099 \pm 0.003	0.104 \pm 0.002	0.099 \pm 0.003
	Excreted (g/7d/rat)	0.078 \pm 0.003	0.082 \pm 0.002	0.077 \pm 0.004
	Retention (g/7d/rat)	0.021 \pm 0.002	0.022 \pm 0.002	0.023 \pm 0.002
	Apparent absorption (%)	39.0 \pm 1.6	38.6 \pm 1.6	40.0 \pm 2.1
Na	Intake (g/7d/rat)	0.204 ^a \pm 0.006	0.228 ^b \pm 0.005	0.223 ^{ab} \pm 0.006
	Excreted (g/7d/rat)	0.178 \pm 0.007	0.186 \pm 0.010	0.168 \pm 0.007
	Retention (g/7d/rat)	0.025 ^a \pm 0.008	0.045 ^{ab} \pm 0.008	0.054 ^b \pm 0.005
	Apparent absorption (%)	97.4 \pm 0.2	97.5 \pm 0.2	97.0 \pm 0.2
K	Intake (g/7d/rat)	0.366 \pm 0.012	0.394 \pm 0.009	0.378 \pm 0.011
	Excreted (g/7d/rat)	0.306 \pm 0.016	0.307 \pm 0.018	0.302 \pm 0.016
	Retention (g/7d/rat)	0.050 \pm 0.015	0.083 \pm 0.015	0.074 \pm 0.009
	Apparent absorption (%)	96.4 \pm 0.3	96.0 \pm 0.3	95.5 \pm 0.4
Fe	Intake (mg/7d/rat)	6.93 \pm 0.22	7.23 \pm 0.15	7.44 \pm 0.22
	Excreted (mg/7d/rat)	15.00 \pm 1.48	16.24 \pm 1.64	18.39 \pm 1.73
	Retention (mg/7d/rat)	-11.12 \pm 3.31	-9.05 \pm 1.63	-11.07 \pm 1.63
	Apparent absorption (%)	-8.7 \pm 9.2	-6.9 \pm 9.8	13.0 \pm 6.8
Zn	Intake (mg/7d/rat)	3.40 ^a \pm 0.11	3.42 ^a \pm 0.08	2.87 ^b \pm 0.13
	Excreted (mg/7d/rat)	5.02 \pm 0.21	4.29 \pm 0.24	4.94 \pm 0.29
	Retention (mg/7d/rat)	-1.55 ^{ab} \pm 0.21	-0.89 ^a \pm 0.23	-2.03 ^b \pm 0.20
	Apparent absorption (%)	-32.0 ^{ab} \pm 8.0	-12.5 ^a \pm 5.8	-50.8 ^b \pm 7.4
Cu	Intake (mg/7d/rat)	0.539 ^a \pm 0.017	0.453 ^b \pm 0.010	0.485 ^b \pm 0.014
	Excreted (mg/7d/rat)	0.509 \pm 0.075	0.486 \pm 0.031	0.396 \pm 0.018
	Retention (mg/7d/rat)	0.041 \pm 0.067	-0.040 \pm 0.029	0.067 \pm 0.019
	Apparent absorption (%)	24.6 ^a \pm 9.9	4.2 ^b \pm 7.1	27.7 ^a \pm 4.1

^{a,b} – means followed by the different superscript differ at $p \leq 0.05$ (ANOVA, Scheffe test)

Summary

Effect of conventional and transgenic maize (Roundup Ready - RR), with an introduced gene of glyphosate resistance (two lots from Monsanto, USA) on minerals was studied in 30 rats (75g) divided into three feeding groups. Ca, Mg, K and Fe intake, apparent absorption and retention did not differ between groups. Higher P retention and absorption was stated only in one group RR2 ($p \leq 0.05$). The Zn apparent absorption was lower ($p \leq 0.05$) in RR2 group than in other groups. However Zn retention in RR2 and control groups were similar, and in both lower than in RR1 group. Although similar Cu intake in all groups, the significantly lower apparent Cu absorption in RR1 group than in the others was observed, however the Cu retention did not differ between groups.

References: The literature is available with the author.

Einsatz von Immunmilchpräparaten als Biestmilchersatz in der Paratuberkulosesanierung

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Einleitung

Bei der Paratuberkulose handelt es sich um eine chronische Durchfallerkrankung, welche unter anderem über die Biestmilchaufnahme auf die Kälber übertragen wird. Ziel unterschiedlicher Sanierungsverfahren ist es u.a. dieses Infektionsrisiko über die Biestmilch zu reduzieren.

Im Rahmen einer Diplomarbeit wurden auf einem mit Paratuberkulose infizierten Milchviehbetrieb mit 200 Kühen in Thüringen Fütterungsversuche mit einem Immunmilchpräparat der Firma Phytobiotics, Eltville, an 29 Kälbern durchgeführt.

Ziel dieser Arbeit war es festzustellen, ob ein vollständiger Ersatz der Biestmilch durch Immunmilchpräparate im Rahmen einer Paratuberkulosesanierung möglich ist.

Material und Methoden

Bei der „Immunmilch“ handelt es sich um ein gefriergetrocknetes, entkeimt- und entfettetes Milchpulver, welches aus dem ersten Gemelk IBR-freier Kühe hergestellt wird. Die „Immunmilch 30“ ist reich an Rohprotein (mind. 75%) und Immunglobulinen (mind. 30%).

Für den Fütterungsversuch wurden insgesamt 20 weibliche Kälber mit der „Immunmilch 30 IBR-frei“ der Firma Phytobiotics in zwei unterschiedlichen Varianten gefüttert, acht Kälber in der Variante 1 und zwölf Kälber in der Variante 2.

Den Tieren der beiden Versuchsgruppen wurden in den ersten 6 Lebensstunden entweder 1,5 oder 3g Immunglobuline je kg Lebendmasse über das Produkt Immunmilch verabreicht. So erhielt je nach Versuchsvariante beispielsweise ein 40kg schweres Kalb 200g bzw. 400g des Produktes (Tab.1).

Die neun männlichen Kälber einer dritten Versuchsgruppe (VB) wurden mit 2,5 L Biestmilch des jeweiligen Muttertieres getränkt. Vor dem Verfüttern wurde die Biestmilch gespindelt, um den Immunglobulingehalt der Milch festzustellen. Die Spindelwerte der verfütterten Biestmilch lagen zwischen 50 und 120g/L.

Tabelle 1: Dosiertabelle „Immunmilch 30 IBR-frei“

Kälbergewicht (kg)	Variante A		Variante B	
	IG- Gehalt (g)	Immunmilch (g)	IG- Gehalt (g)	Immunmilch (g)
35	53	175	105	350
40	60	200	120	400
45	68	225	135	450
50	75	250	150	500
55	83	275	165	550

IG = Immunglobulin

48 h nach der Geburt wurden allen Kälbern Blutproben entnommen und beim Tiergesundheitsdienst Thüringen e.V. auf den Eiweiß- und Gammaglobulingehalt untersucht. Zudem wurde in den ersten 14 Lebenstagen die Entwicklung (durchschnittliche Tageszunahmen) und der Gesundheitsstatus der Kälber (Tage mit Durchfallerkrankung) dokumentiert.

Ergebnisse

Die Versuchsauswertung ergab, dass sich die beiden unterschiedlichen Immunglobulindosierungen in den Blutwerten der Kälber nur gering widerspiegeln, der Unterschied zu den Blutwerten der mit Biestmilch gefütterten Bullenkälber dagegen war signifikant (Tab. 2).

Tabelle 2: Ergebnisse Eiweiß- und Gammaglobulingehalt im Blut

	Variante A	Variante B	Biestmilch
Eiweißgehalt im Blut (g/L)	47 ^B	49 ^B	67 ^A
Gammaglobulingehalt im Blut (g/L)	11 ^B	13 ^B	28 ^A

Für den Eiweißgehalt im Blut wurde vom Tiergesundheitsdienst Thüringen e.V. ein Mindestwert von 53 g/L Blut angegeben. Beide Immunmilchvarianten erreichen diesen Wert mit 47 g/L bei der Variante 1 und 49 g/L bei der Variante 2 nicht. Einzig die Bullenkälber liegen mit 67 g/L deutlich über dem geforderten Mindestwert (Abb. 1).

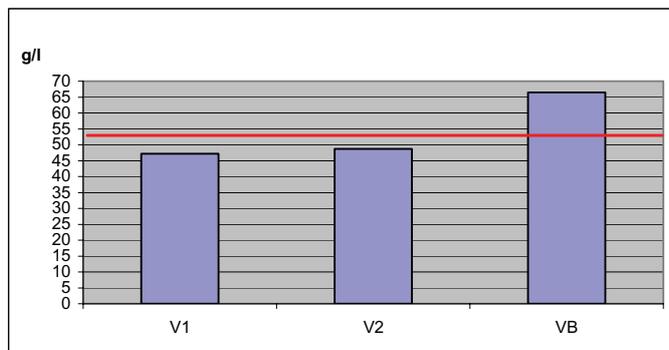


Abbildung 1: Eiweißgehalt im Blut

Hinsichtlich der Gammaglobulingehalte ergibt sich ein ähnliches Bild. Hier gibt der Tiergesundheitsdienst Thüringen e.V. einen Mindestwert von 12 g/L Blut an, die beiden Immunmilchvarianten bewegen sich mit 11 g/L bei der Variante 1 und 13 g/L bei der Variante 2 knapp unter bzw. in dem Mindestbereich. Die Bullenkälber liegen auch hier mit 28 g/L deutlich über dem geforderten Mindestwert (Abb. 2).

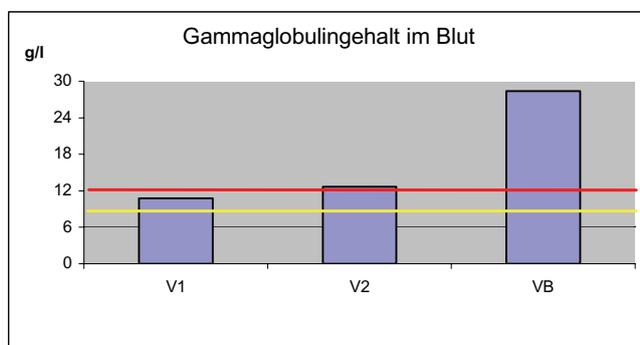


Abbildung 2: Gammaglobulingehalt im Blut

Andere Literaturangaben fordern nur einen Gammaglobulingehalt als Mindestwert von 8 g/L Blut (TRILK et al. 2000). Damit verglichen liegen beide Immunmilchvarianten darüber und weisen eine ausreichende Gammaglobulinversorgung auf. Die Ergebnisse der Lebendmasseentwicklung und der dokumentierte Gesundheitsstatus

der Kälber bestätigen diesen niedriger angesetzten Mindestwert von 8 g Gammaglobulin/L Blut (Tab. 3).

Tabelle 3: Ergebnisse Tageszunahmen und Durchfallerkrankung

	Variante A	Variante B	Biestmilch
Durchschnittliche Tageszunahmen (g/Tag)	634 ^B	744 ^B	929 ^A
Durchschnittliche Durchfallerkrankung (Tage)	1,1 ^A	0,9 ^A	0,4 ^B

Die durchschnittlichen Tageszunahmen der Biestmilchkälber sind zwar signifikant höher als die der beiden Immunmilchvarianten, jedoch dürfte dieser Effekt geschlechtsspezifisch zu interpretieren sein, da für die Biestmilchgruppe nur Bullenkälber verwendet werden konnten.

Auch der signifikante Unterschied in der durchschnittlichen Anzahl der Durchfalltage pro Kalb zwischen den Varianten sollte aufgrund des insgesamt sehr niedrigen Niveaus von unter 0,8 Tagen pro Kalb nicht überbewertet werden. Insgesamt zeigte sich bei den Tieren im Versuchsstadium ein sehr guter Gesundheitszustand.

Fazit

Der Versuch hat ergeben, dass ein vollständiger Ersatz der Biestmilch durch die „Immunmilch 30“ möglich ist.

Trotz der relativ geringen Gammaglobulin- und Eiweißgehalte im Blut der Immunmilchkälber konnten diese eine befriedigende Gewichtsentwicklung und einen guten Gesundheitsstatus aufweisen. Es bleibt zu diskutieren, wo der Mindestgammaglobulingehalt im Blut anzusetzen ist.

Im Rahmen einer Paratuberkulosesanierung vermindert der Einsatz von „Immunmilch 30“ als vollständiger Biestmilchersatz das Paratuberkulose-Infektionsrisiko der Kälber.

Literatur

Trilk et al. (2000) : *Zusammenhänge zwischen Kälbergesundheit und Wachstumsverlauf bei Jungrindern*, Kälber und Jungrinderaufzucht, Deutsche Gesellschaft für Züchtungskunde (Hrsg.), Bonn, Seite 52

The effect of benzoic acid on growth performance, digestibility of nutrients, nitrogen balance and gastro-intestinal microflora in piglets

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Introduction

Benzoic acid is one of the oldest chemical preservatives used in food for human consumption. Benzoate inhibits yeasts more than bacteria. In undissociated form benzoic acid is soluble in cell membranes and facilitates proton leakage into cells, increasing cellular energy requirements to maintain internal pH. The undissociated form is essential to its antimicrobial activity. It has been shown that benzoic acid also reduces growth of microorganisms in the intestinal digesta of piglets (Knarrenborg et al. 2002). This suggests that benzoic acid could favourably influence the digestibility of nutrients and growth parameters of piglets. The aim of this study, therefore, was to investigate the effect of benzoic acid as a feed additive on growth performance, digestibility of nutrients, gastro-intestinal microflora and urine pH in piglets.

Materials and Methods

In total 54 hybrid piglets of 28-days at weaning were used in the performance experiment. They were divided into three groups of 18 animals each. Nine replicates of 2 piglets were assigned to each dietary treatment. The animals assigned to treatment 1 received the basal diet consisting of 51 % wheat, 20,3 % barley, 23 % soybean meal, 2,5 % soybean oil and 3,2 % mineral vitamine premix. Animals of treatment 2 received the basal diet supplemented with benzoic acid at 5 g/kg (VevoVital[®], DSM) and animals of treatment 3 received the diet supplemented with benzoic acid at 10 g/kg. The feeding period lasted over 35 days. The balance experiment was conducted with 18 crossbreed castrates. The animals were allotted to three groups of six each. The composition of the diets was identical with that used in the performance experiment, but different batches of ingredients were used for the preparation of this diets. At an average body weight of 16.2 kg the pigs were transferred into metabolic cages. Faeces and urine were collected for a period of six days. At the end of the collection period, the piglets were slaughtered and the gastro-

intestinal tract and the urinary bladder were immediately removed to measurement the pH value and bacterial count by standard methods (Balows et al. 1991).

Results

In the performance experiment, piglets fed the diet with 1.0 % benzoic acid had significantly higher daily weight gains (+ 15 %, $P < 0.05$) than piglets fed the control diet. Piglets fed diets supplemented with either 0.5 % or 1.0 % benzoic acid showed a tendency ($P < 0.10$) towards higher feed intake and final body weight at day 35 compared to the control piglets fed the unsupplemented diet (Table 1). Benzoic acid caused also a slight dose-related improvement of the feed conversion (by 1.9 and 4.4%), although the difference was not statistically significant.

Table 2. Body weights, body weight gains, feed intake and feed conversion of piglets fed a control diet or diets supplemented with benzoic acid (5 or 10 g/kg)

Diet	1	2	3
Benzoic acid (g/kg)	0	5	10
Body weight (kg)			
Initial	7.3 ± 0.7	7.3 ± 0.4	7.3 ± 0.3
Day 35	19.1 ± 1.7	20.3 ± 2.3	20.8 ± 1.8
Body weight gain (g/d)	338 ± 46 ^a	374 ± 58 ^{ab}	387 ± 51 ^b
Feed intake (g/d)	532 ± 71	577 ± 83	586 ± 71
Feed conversion (feed:gain)	1.58 ± 0.09	1.55 ± 0.05	1.51 ± 0.09
Means ± SD			
^{a,b} Means not sharing the same superscript letter are significantly different ($P < 0.05$).			

Dietary supplementation with benzoic acid did not significantly influence the faecal digestibility of total organic matter, crude protein, crude fat, crude fibre and N-free extract (Table 3), although some numerical improvements were noted. However, piglets receiving benzoic acid at 0.5 % or 1.0 % showed a significantly higher absolute nitrogen retention than piglets fed the control diet (Table 2).

The pH of the digesta in the stomach, duodenum, jejunum and ileum was not altered by dietary supplementation with benzoic acid while the urinary pH was reduced by benzoic acid in a dose-dependent manner (Table 3).

Table 2. Apparent faecal digestibility of nutrients and nitrogen balance in piglets fed a control diet or diets supplemented with benzoic acid

Diet	1	2	3
Benzoic acid (g/kg)	0	5	10
Apparent digestibility (%)			
Organic matter	90.3 ± 1.7	91.9 ± 1.5	91.1 ± 1.5
Crude protein	88.1 ± 3.6	89.7 ± 2.4	88.8 ± 1.7
Crude fat	84.0 ± 2.6	86.4 ± 2.6	84.7 ± 2.8
Crude fibre	47.5 ± 10.0	57.8 ± 8.2	52.0 ± 8.1
N-free extract	93.3 ± 0.6	95.0 ± 1.0	94.5 ± 0.7
Nitrogen balance			
Nitrogen intake (g/d)	23.71 ± 1.10	23.84 ± 1.05	24.26 ± 1.05
Nitrogen excretion via faeces (g/d)	2.82 ± 0.85	2.47 ± 0.63	2.71 ± 0.40
Nitrogen excretion via urine (g/d)	5.32 ± 1.38	5.05 ± 0.90	5.09 ± 0.47
Nitrogen retention (g/d)	15.57 ± 0.46 ^a	16.33 ± 0.38 ^b	16.46 ± 0.51 ^b
Means ± SD			
^{a,b} Means not sharing the same superscript letter are significantly different (P<0.05).			

Table 3. pH values of the digesta of stomach, duodenum, jejunum and ileum and in the urine of piglets fed a control diet or diets supplemented with benzoic acid

Diet	1	2	3
Benzoic acid (g/kg)	0	5	10
Digesta			
Stomach	4.40 ± 0.16	4.21 ± 0.35	4.21 ± 0.15
Duodenum	5.68 ± 0.11	5.64 ± 0.24	5.51 ± 0.21
Jejunum	6.14 ± 0.21	6.10 ± 0.39	6.15 ± 0.09
Ileum	6.55 ± 0.36	6.37 ± 0.44	6.66 ± 0.07
Urine	7.45 ± 0.19 ^a	6.87 ± 0.54 ^{ab}	6.42 ± 0.59 ^b
Means ±SD			
^{a,b} Means not sharing the same superscript letter are significantly different (P<0.05).			

The addition of benzoic acid also resulted in a dose-dependent reduction of total aerobic and anaerobic bacteria and lactic acid bacteria in the stomach (Table 4). The number of aerobic, anaerobic and lactic acid bacteria in the duodenum was not

influenced by dietary supplementation with benzoic acid while gram-negative bacteria were reduced by benzoic acid in a dose-dependent manner.

Table 4. Count of total aerobic, total anaerobic, lactic acid bacteria and gram-negative bacteria in the digesta of stomach, duodenum, of piglets fed a control diet or diets supplemented with benzoic acid

Diet	1	2	3
Benzoic acid (g/kg)	0	5	10
Count (log 10/g fresh mater of digesta)			
Stomach			
Total aerobic bacteria	9.06 ± 1.99 ^a	7.47 ± 1.76 ^{ab}	5.71 ± 0.62 ^b
Total anaerobic bacteria	9.22 ± 1.99 ^a	7.79 ± 2.18 ^{ab}	5.67 ± 0.40 ^b
Lactic acid bacteria	8.87 ± 2.05 ^a	7.61 ± 1.94 ^{ab}	5.48 ± 0.63 ^b
Gram-negative bacteria	< 3.0	< 3.0	< 3.0
Duodenum			
Total aerobic bacteria	10.07 ± 0.93	9.97 ± 0.95	10.33 ± 0.38
Total anaerobic bacteria	10.43 ± 0.42	10.42 ± 0.96	10.32 ± 0.57
Lactic acid bacteria	9.12 ± 1.66	9.82 ± 1.65	9.91 ± 1.12
Gram-negative bacteria	7.27 ± 2.73 ^a	4.95 ± 2.32 ^{ab}	3.97 ± 0.88 ^b
Means ±SD			
^{a,b} Means not sharing the same superscript letter are significantly different (P<0.05).			

Summary

In order to investigate the effects of benzoic acid (0.5 % and 1.0 % in the diet) on growth performance, nutrient digestibility, nitrogen balance and gastrointestinal microflora of piglets, we conducted a performance experiment and a separate balance study. The data of this study suggest that benzoic acid exerts strong antimicrobial effects in the gastrointestinal tract of piglets and therefore enhances growth performance and nitrogen retention.

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Untersuchungen zum Einfluss von Kreatin auf das Wachstum und die Ganzkörperzusammensetzung von Broilern

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Einleitung

Kreatin kommt als körpereigene Substanz im menschlichen und tierischen Körper vor. Durch Eigensynthese wird ein Teil der täglich benötigten Menge gebildet, ein weiterer Teil kann mit der Nahrung aufgenommen werden. Die bedeutendsten Kreatinlieferer sind Muskelfleisch und Fisch. Kreatin spielt eine wichtige Rolle beim Energietransport in der Skelett-Muskulatur, weshalb die Substanz teilweise im Sport zur Steigerung der Muskelkraft eingesetzt wird (Mertschenk u. a., 2001). In der Tierernährung liegen wenige Erfahrungen zur Bedeutung von Kreatin als Futterbestandteil vor. Erste Ergebnisse zum Einfluss der Supplementierung von Kreatin ins Broilermastfutter wurden 2003 (Halle) vorgestellt.

Das Ziel dieser Untersuchungen bestand darin den Einfluss von Kreatin im Mastfutter auf Wachstum und Schlachtkörpermerkmale von Broilern zu prüfen.

Material und Methoden

In den Versuchen 1 und 2 wurden 350 bzw. 210 männliche Broiler der Herkunft Lohmann Meat und im Versuch 4 180 Küken der Herkunft ISA 257 als Eintagsküken in eine Mastanlage eingestallt. Jede Gruppe umfasste 10 Wiederholungen. Im Versuch 3 wurden 336 Küken (Lohmann Meat) auf eine Kontroll- und zwei Versuchsgruppen mit jeweils 8, 7 oder 6 Tieren pro Käfig aufgeteilt. Die Prüfperiode umfasste in den Versuchen 1 - 3 35 Tage und im Versuch 4 84 Tage. Am Versuchabschluss wurde jeweils ein Broiler, dessen Gewicht dem Mittelwert des Käfigs der Gruppe entsprach, ausgeschlachtet. Die Zusammensetzung der Futtermischungen ist aus Tabelle 1 abzulesen. Die statistische Auswertung der Merkmale erfolgte unter Verwendung des Programmpaketes SAS 9.1. Der Mittelwertvergleich wurde mit dem Student-Newman-Keuls-Test ($P \leq 0,05$) durchgeführt.

Ergebnisse

Während im ersten Broilermastversuch über 35 Tage die Lebendmassezunahme der Tiere, deren Futter Kreatin enthielt, statistisch gesichert ($P < 0,05$) höher war (4,5 –

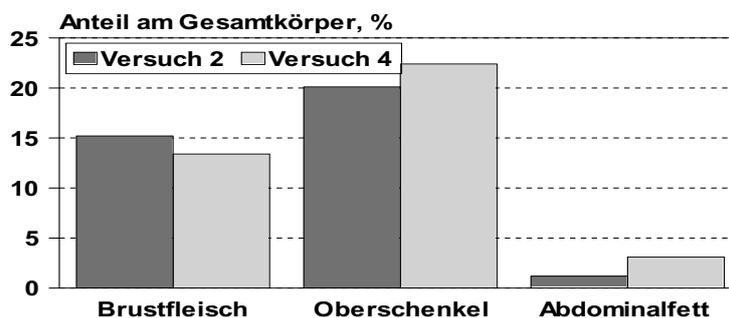
6,9 %) als bei den Kontrolltieren, konnte dieses Ergebnis in den Wiederholungsversuchen 2 und 3 nicht bestätigt werden (Tab. 2). Bei einer differenzierten Anzahl an Broilern pro Käfig (Versuch 3) verbesserte sich die Futterraufnahme insbesondere durch einen sinkenden Tierbesatz und resultierend daraus war die tägliche Lebendmassezunahme in diesen Gruppen besser. Die Supplementierung von 1 g Kreatin pro kg Futter während einer Mastdauer von 84 Tagen verschlechterte gesichert die tägliche Lebendmassezunahme der langsam wachsenden Broiler.

Tabelle 1: Zusammensetzung und wert bestimmende Inhaltsstoffe (g/kg)

	Versuch 1 - 3	Versuch 4 P1 1.-14. Tg.	P2 15.-56.Tg.	P3 57.-84. Tg.
Weizen+Mais	553	553	620	644
Soja	373	373	303	276
Sojaöl	29	29	33	37
Mineralstoffe+Vitamine	41	41	40	40
Methionin+Lysin	4	4	4	3
Kreatinmonohydrat	0/0,5/1/2/5/10	0/0,5/1	0/0,5/1	0/0,5/1
Rohprotein	21,5	21,5	19,5	18,5
ME, MJ/kg	12,8	12,8	13,0	13,2

Die Ausschachtung der Broiler am 35. bzw. 84. Lebenstag ergab keine Unterschiede in den Anteilen an Brustmuskel und Oberschenkelmuskel sowie den Organen und Abdominalfett zwischen der Kontrolle und den Versuchsgruppen der Versuche 1 - 4.

Abbildung 1: Ausgewählte Ausschachtungsergebnisse nach 35 bzw. 84 Tagen



In der Abbildung 1 werden die Anteile an Brust-, Oberschenkelfleisch und Abdominalfett der Broiler der Kontrollgruppe des Versuches 2 nach einer Mastdauer von 35 Tagen verglichen mit den Broilern, die 84 Tage (Versuch 4) gemästet wurden.

Tabelle 2: Futteraufnahme (FA, g/Tier/Tag) Lebendmassezunahme (LMZ, g/Tier) und Futteraufwand (FAW, kg/kg) der Broiler in den Wachstumsversuchen (Mittelwert +Standardabweichung)

Versuch 1	Kontrolle	1g Kreatin	2g Kreatin	5g Kreatin	10g Kreatin
FA	89 ±2	93 ±1	92 ±3	93 ±5	91 ±4
LMZ	2092b ±184	2237a ±162	2215a ±205	2229 a ±191	2187 a ±189
FAW	1,521 ±0,05	1,479 ±0,03	1,474 ±0,06	1,480 ±0,06	1,478±0,05
Versuch 2	Kontrolle	0,5g Kreatin	1g Kreatin	-	-
FA	100 ±3	98 ±3	97 ±4		
LMZ	2329 ±69	2320 ±70	2280 ±123		
FAW	1,512 ±0,02	1,502 ±0,03	1,536 ±0,07		
Versuch 3	Kontrolle	0,5g Kreatin	-	-	-
FA					
8 Tiere	95 ±3	94 ±5			
7 Tiere	95 ±3	98 ±3			
6 Tiere	98 ±4	96 ±3			
LMZ					
8 Tiere	2188 ±58	2197 ±100			
7 Tiere	2189 ±49	2253 ±44			
6 Tiere	2275 ±58	2251 ±71			
FAW					
8 Tiere	1,520 ±0,03	1,498 ±0,09			
7 Tiere	1,518 ±0,04	1,528 ±0,03			
6 Tiere	1,510 ±0,03	1,499 ±0,04			
Versuch 4	Kontrolle	0,5g Kreatin	1g Kreatin		
FA	110 ±6,4	108 ±6	108 ±4		
LMZ	4281a±160	4173ab ±199	4081b±162		
FAW	2,160 ±0,06	2,176 ±0,06	2,217±0,08		

a, b – signifikante Unterschiede zwischen den Gruppen bei einem Merkmal

Während die Broiler in der Kurzmast einen höheren Anteil an Brustfleisch bildeten, war für eine gute Beweglichkeit der Broiler der Langmast eine intensivere Ausbildung der Oberschenkelmuskulatur notwendig. Allerdings stieg der Anteil an Abdominalfett während der Langmast auf über 3 % an.

Schlussfolgerungen

Die Ergebnisse lassen keine eindeutige Schlussfolgerung zu, dass die Substanz Kreatin das Wachstum von Broilern als auch den Futteraufwand während einer Kurzmast positiv beeinflussen kann. Eine Dosis-Wirkungsbeziehung zwischen den Kreatingruppen wurde nicht ermittelt. Die Supplementierung von Kreatin in das Mastfutter der Broiler während der Langmast verschlechterte die Futteraufnahme der Tiere, insbesondere nach dem 35. Masttag, was sich negativ auf die Mastendmasse auswirkte.

Summary

Studies on the effect of Creatine on growth performance and on carcass quality of broiler chickens

Creatine is a substance occurring naturally in the animal body. The major proportion of the total Creatine pool is found in skeletal muscle. The present four studies were focused on the observation of the growth performance of male growing broiler chickens in dependence on Creatine feeding. Influences on carcass quality were investigated at 35 days (Experiments 1, 2, 3) or 84 days (Experiment 4) of age. In Experiment 1, Creatine showed significant growth promoting effects. In Experiment 2 and 3 Creatine supplementation didn't change final live weight at day 35 of age. In Experiment 4 final body weight was depressed after 1 g Creatine supplementation at day 84 of age. No effects on the carcass quality of broilers could be shown in the four Experiments.

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Untersuchungen zum Einfluss von Kreatin auf die Leistungsmerkmale von Legehennen

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Einleitung

Kreatin kommt als körpereigene Substanz im menschlichen und tierischen Körper vor, weshalb die bedeutendsten Kreatinlieferer Muskelfleisch und Fisch sind. Ein Teil der täglich benötigten Menge an Kreatin wird durch Eigensynthese in Leber, Nieren und Pankreas gebildet, der andere Teil wird mit der Nahrung aufgenommen. Bei Vegetariern und Veganern, die über ihre Nahrung kaum Kreatin aufnehmen, ist der Kreatingehalt im Serum über 30 % geringer als bei Menschen mit normaler Ernährung (Krämer et al., 2001).

Resultierend aus dieser Tatsache und dem bestehenden Verbot der Verfütterung von Tier- und Fischmehlen als Futtermittel in der Geflügelfütterung, ergab sich die Frage, ob eine Anreicherung des Hennenfutters mit Kreatin die Legeintensität und das Eigewicht sowie die Anzahl befruchteter Eier und geschlüpfter Küken beeinflussen kann.

Material und Methoden

144 Legehybriden (LSL) wurden in 4 Gruppen a 36 Hennen aufgeteilt und in Einzelhaltung in einer Mehretagenbatterie untergebracht. Die Käfige waren mit Nippeltränken und Einzelfuttergefäßen versehen. Mit Beginn der 23. Lebenswoche der Hennen wurde der Versuch gestartet. Er umfasste einen Zeitraum von 13 Legemonaten. Während der Legeperiode wurden die täglich gelegten Eier von jeder Henne registriert. Die Einzeleimasse wurde an vier Tagen in zwei Wochen in jedem Legemonat ermittelt. Die Hennen wurden künstlich besamt und alle Eier aus der 30., 45. und 65. Lebenswoche der Hennen in einen Brutapparat eingelegt. Das Futter (Tab. 1) wurde zur freien Aufnahme angeboten und die Futterrückwaage einmal monatlich durchgeführt.

Die statistische Auswertung der Merkmale erfolgte unter Verwendung des Programmpaketes SAS (Version 9.1). Signifikante Unterschiede in den Leistungsmerkmalen zwischen den Gruppen wurden über den multiplen Mittelwertvergleich Student-Newman-Keuls-Test ($P \leq 0,05$) errechnet.

Tabelle 1: Zusammensetzung und wertbestimmende Inhaltsstoffe der Futtermischungen (g/kg)

Komponenten	Kontrolle
Weizen/Gerste/Mais	717
Soja/Sojaöl	167
Mineralstoffe/Vitamine	113
Methionin	3
Kreatinmonohydrat	0/0,5/1/2
Rohprotein	155
ME, MJ/kg	10,8

Ergebnisse

Die gestaffelte Supplementierung von Kreatin ins Hennenfutter führte zu einer erhöhten Futteraufnahme, aber nicht in allen drei Gruppen zu einer statistisch gesicherten ($P < 0,05$) Verbesserung (Tab. 2).

Die Legeintensität lag im Mittel der 13 Monate zwischen 92 und 93 % und war zwischen den Gruppen gleich.

Auch die Einzeleimasse von 60 – 61 g war bei allen Gruppen gleich. Resultierend aus den beiden Einzelergebnissen war auch die errechnete tägliche Eimasseproduktion ohne Unterschied zwischen Kontrolle und Versuchsgruppen. Der Futteraufwand von 2,06 kg Futter pro kg Eimasse der Gruppe mit 2 g Kreatin im Futter war gesichert höher als der, der Hennen der Kontrolle.

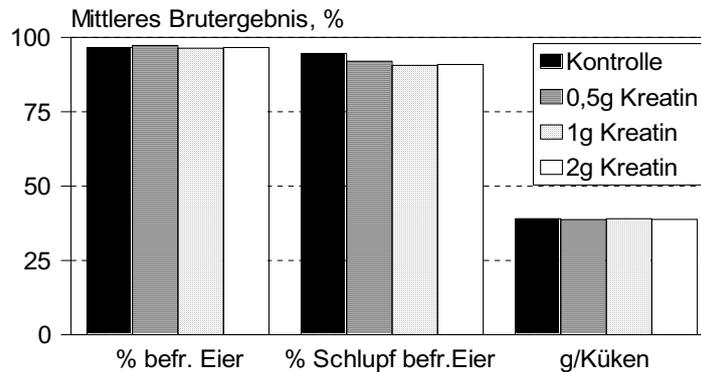
In den drei Brutversuchen wurde kein Unterschied zwischen den Gruppen in der Anzahl an befruchteten Eiern, in der Anzahl geschlüpfter Küken aus den befruchteten Eiern sowie im Kükengewicht ermittelt. In der Abbildung 1 sind die Mittelwerte der genannten aus den drei Brutversuchen dargestellt.

Tabelle 2: Legeleistungsmerkmale im Mittel der 13 Legemonate
(Mittelwert \pm Standardabweichung)

	Kontrolle	Kreatin 0,5 g	Kreatin 1,0 g	Kreatin 2,0 g
Futtermittelaufnahme, g/Tier und Tag	109,9 b \pm 10,6	113,1 a \pm 11,4	111,2 b \pm 11,9	113,3a \pm 13,2
Legeintensität, %	92,6 \pm 10,6	93,4 \pm 10,9	93,5 \pm 11,6	92,3 \pm 12,3
Eimasse, g/Ei	60,4 \pm 4,9	60,6 \pm 5,1	60,4 \pm 5,8	61,0 \pm 6,1
Eimasseproduktion, g/Henne und Tag	56,0 \pm 8,1	56,8 \pm 8,8	56,7 \pm 9,4	56,6 \pm 10,0
Futtermittelaufwand, kg/kg	1,994 b \pm 0,28	2,031ab \pm 0,31	2,011ab \pm 0,38	2,060 a \pm 0,40

a; b – signifikante Unterschiede zwischen den Gruppen bei einem Merkmal

Abbildung 1: Brutergebnisse (Mittelwerte aus drei Brutversuchen)



Schlussfolgerungen

Die gestaffelte Supplementierung von Kreatin in das Legehennenfutter erhöhte die tägliche Futtermittelaufnahme der Hennen, ohne dass die verbesserte Nährstoffaufnahme in einer Leistungssteigerung bemerkbar wurde. Auch die Zuchtmerkmale wurden nicht verändert. Dabei ist allerdings festzustellen, dass die Leistungen der Hennen insgesamt schon sehr hoch waren und deshalb eine Leistungsverbesserung kaum

noch möglich war. Die gesteigerte Futtermittelaufnahme durch die Kreatinzufuhr im Futter ist ein Hinweis, dass unter stärkerer Belastung (z. B. Gruppen- und Auslaufhaltung, Infektionsdruck) die positive Wirkung von Kreatin stärker zum Ausdruck kommen kann.

Summary

Studies on the effect of Creatine on performance of laying hens

Creatine is a substance occurring naturally and is most abundant in fish and meat. The situation that the feeding of animal meat meal to hens is forbidden, the question arose whether a supplementation of Creatine to diet improves the performance of breeding hens. A total of 144 laying hybrids were allocated to 4 groups with 36 hens per group. The basal diet was formulated to contain 0/0.5/1/2 g Creatine per kg. The duration time of the trial was 13 month. Hens were artificial inseminated; eggs were stored in the incubator at a hen age of 30, 45 and 65 weeks. The laying intensity and egg mass production of hens fed the diets, supplemented with Creatine, were not improved. Neither hatchability nor chickens weight at hatching were significantly affected by Creatine supplementation.

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Investigations on the use of trace elements for authentication of the origin of poultry and beef

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Introduction

Consumer demands for authenticity of the origin of meat are rapidly increasing. For 82% of the customers, origin of food is important for their purchase decision (Anonymous, 2004a). Therefore, sophisticated analytical tools are required to prove the origin of food, a result being valuable also to organizations controlling stated origins of the products and to producers. For dried beef from the Swiss canton of Valais, for instance, producers claim the exclusive use of Swiss raw beef (Anonymous, 2002), different from producers of such products in canton Grisons ('Bündnerfleisch'). The country of origin is of great interest for poultry, too, regarding the current avian influenza situation, for example.

Trace elements might help to discriminate between different origins of meat. Rare elements and those not commonly supplemented to animal diets seem to be promising in that respect since they are more likely to be region-specific (Franke et al., 2005). For example the Se content of American soils is known to be much higher than that in Europe, and the isotopic ratio of Sr is known to be influenced by the type of geological underground (Capo et al., 1998). Also Rb is of interest as it was found to be enriched in granite and gneiss weathering soil (Anke and Angelow, 1995). Another good option to prove origin is seen in Lanthanides because they are used for decades in certain countries (e.g. China) in pig fattening as growth promoters (Eisele, 2003).

Material and Methods

In order to test the potential of using trace elements to authenticate the geographic origin of meat, two different commodities were selected. The first one was poultry, the most imported meat type in Switzerland (Anonymous, 2004b), where production factors (feed, genotype) with the exception of drinking water and litter are widely globalized and therefore uncoupled from regional origin. The second commodity was

a high-priced dried beef product where the production of the raw beef depends more on geographic origin but where the trace element profile might also be influenced by transformation from raw meat into the final product (curing salts, herbs etc.).

Poultry breasts from Thailand (n=3), France (n=2), Germany (n=3), Hungary (n=6), Brazil (n=4) and Switzerland (n=7) were obtained. The authenticity of the samples was confirmed by official customs documents where the abattoir was stated. The dried beef meat samples originated from Austria (n=2), Australia (n=1), Canada (n=2) and Switzerland (Valais (n=3) and Grisons). The samples from Grisons were subdivided into raw beef origins from Switzerland (n=4) and Brazil (n=4). Furthermore, two Swiss produced samples of Bresaola, another type of dried beef, were obtained, one prepared from Swiss and one from Brazilian raw meat. All dried beef samples were either collected at the point of production in Switzerland or imported directly from the producers abroad.

Poultry samples were deep frozen and beef samples were stored at +5°C in vacuumized plastic bags. The poultry samples were homogenized using a Büchi Mixer B 400 (Büchi Labortechnik AG, Flawill, CH) equipped with a ceramic knife. Afterwards, 1 g of poultry and 0.5 g of dried beef respectively were subjected to micro-wave assisted pressure digestion with nitric acid. In order to test the quality of the measurements, reference materials (lyophilized bovine muscle BCR 184, Reference Material 8414 NIST) were digested and analyzed together with the samples, which were analyzed for a total of 75 elements/isotopes using a sector field ICP-MS (Element 2, Finnigan MAT, Bremen, D). Multiple linear regression with backward elimination was performed as discriminant analysis to determine the extent to which the variation among samples was explained by distinct combinations of elements/isotopes.

Results

The samples were grouped into Swiss (x) and non-Swiss (o) categories. In poultry breast, 60% of the total variance ($p < 0.001$) were explained due to B, Ca, Co, Tl. Ca and Tl alone explained 42% of the total variance ($p < 0.01$) (Fig. 1). In beef, 86% of the total variance ($p < 0.05$) of samples made from Swiss raw meat and foreign raw meat were explained by Ca, Cu, Li, Pd, Rb, Sc, Sr, Tl, U and V, while Li, Rb and Tl alone explained 80% of the total variance ($p < 0.001$). With these three elements it was possible to differentiate the samples according to the origin of their raw meat. Rare

earth elements could hardly be detected in any of the samples.

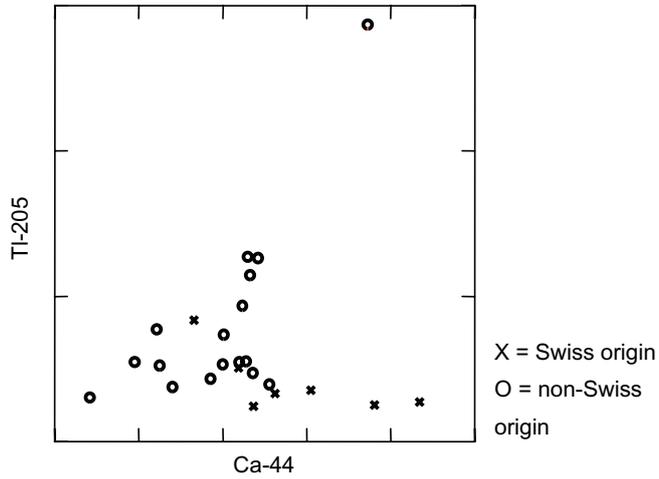


Figure 1. Elements allowing the partial differentiation of poultry meat from Swiss and non-Swiss origin

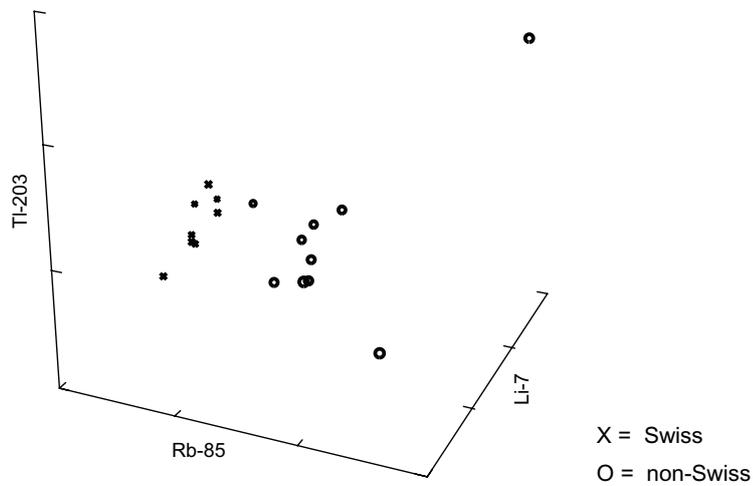


Figure 2. Elements allowing differentiation of dried beef meat produced from Swiss and non-Swiss raw meat

Discussion

The present results show that trace elements can be used to obtain indications of the

geographic origin of poultry breast and dried beef meat. In both cases, few elements/isotopes explained most of the variation. Poultry could not be completely differentiated into two groups (Swiss and non-Swiss origin) when using the elements Tl and Ca, although there were a lot of samples which could be classified correctly. In the case of dried beef, a clear differentiation of Swiss and foreign raw meat was possible. Results obtained with processed meat have to be interpreted carefully because processing might modify the characteristic trace element profile of meat and thus cause a bias with geographic origin. Our results demonstrate a large potential of trace elements for differentiating meat by geographic origin. Combinations with other methods would further improve the accuracy of meat authentication.

Summary

Trace elements, discussed as to be promising to prove geographic origin of meat, were determined in poultry and dried beef. Poultry breasts were obtained from Switzerland and five other countries. Dried beef samples originated from Switzerland (two regions and, in one region, raw beef from Switzerland or Brazil) and three other countries. A total of 75 elements/isotopes were analyzed by using a sector field ICP-MS combined with subjection through micro-wave assisted pressure digestion with nitric acid. A relatively good differentiation of meat from Swiss and from non-Swiss origin was possible with Ca and Tl for poultry and Li, Rb and Tl for dried beef.

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Fruits and vegetables as a source of the mineral elements in the food rations planned for alimentation of young men doing military service in the Polish Army

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Introduction

Fruits and vegetables are an indispensable element of the proper nutrition. They are a main source of vitamins, mineral elements, fiber and other bioactive substances (5). They should be a regular component of daily food rations. Contemporary nutritional knowledge allows saying that fruits, vegetables and their preserves should be included in human's everyday diet. Introduction of these products, fresh or processed, into every meal is important element of proper nutrition (7). Fruits and vegetables add variety to menus by their colour, smell and taste. When planning everyday alimentation one should care not only about presence of fruits and vegetables in the menu but also about their variety (2).

Fruits and vegetables are low-calorie products. They are a source of water-soluble vitamins and mineral elements contained in them alkalize organism and neutralize the excess of acids delivered by other products. They keep the acid-base equilibrium in organism (1). It is considered that regular consumption of fruits and vegetables significantly influence on decrease of nutrition related diseases such as atherosclerosis, cardiovascular system diseases or certain types of neoplasm (4,7). Increase of fresh fruits and vegetables consumption is probably one of the most important dietary factors that has influenced on considerable decrease of gastric carcinoma cases in many countries all over the world including Poland during last several dozen years (10). American recommendations say that 5-9 portions of food eaten every day should contain fresh or processed fruits and vegetables.

One of the tasks of alimentation policies in many countries is change of the nutrition manners to reduce number of factors affecting increase of incidences of so-called non-infectious nutritional dependent diseases among society. Important elements of this strategy are programs promoting increased fruits and vegetables consumption

(3). Low fruits and vegetables consumption level is one of the ten crucial risk factors of diet-dependent diseases development (3).

The aim of the work was analyze of fruits and vegetables intake with daily food rations planned for alimentation of young men doing military service in Polish Army units. Fruits and vegetables participation in delivery of fundamental mineral elements was assessed as well.

Material and Methods

Evaluation of 50 daily food rations planned for soldiers nutrition in winter season was done. Using the calculation software FOOD 2 (8) the sodium, potassium, calcium, phosphorus, iron, zinc, copper and manganese content in vegetables planned in the soldier's ration was estimated.

Results

It was found that during winter season planned daily food ration contained 458,5 g vegetables and 373 g fruits in an average, both fresh and processed. Planned fruits and vegetables were very diversified.

Optimal amounts of fruits and vegetables consumption resulting from the recommendations of the „Proper nutrition pyramid” amounts to approx. 500 g for vegetables and 300 g for fruits daily and should be served in few portions (11).

Planned in the winter season amount of vegetables was a little bit lower while amount of fruits was higher by 25% comparing to the recommendation. It is well known the amount of eaten fruits and vegetables is too low in Poland comparing to the recommended norms (Fig 1) (5). Among countries joining the EU vegetables consumption in Poland was lower by 27,2% than the highest level. Fruits consumption in Poland was lower by 57% comparing to the country of the highest level (6). A poll conducted among students of Medical Academy in Łódź revealed that every fifth men did not eat any meals containing fruits and vegetables (9).

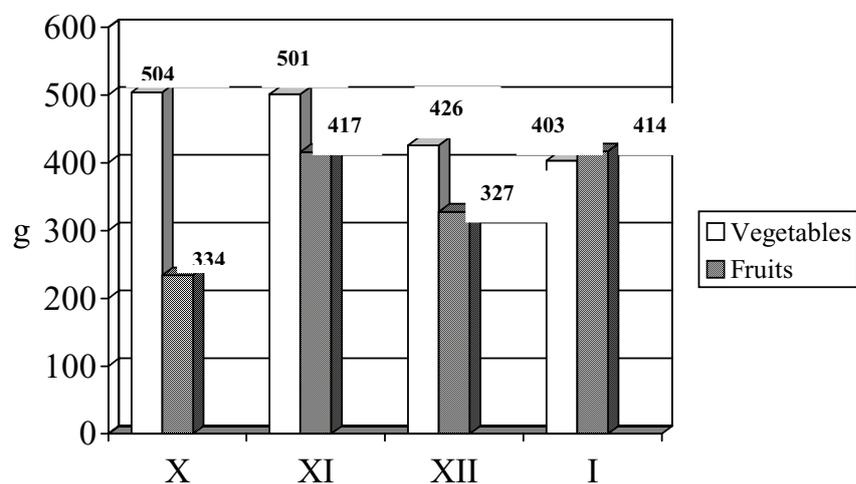


Fig. 1. Fruits and vegetables intake in food rations planned for consumption

It was found that sodium content in vegetables planned in daily ration met the requirements obligatory in Poland in 90%. Manganese met the requirements in 4% only what was the lowest amount. Fruits delivered the biggest amount of potassium meeting the requirements in 22.4% but zinc intake with fruits was the lowest and indicated 3.4%. of the recommended amount (Tabl. 1) (11).

Table 1. Content of selected mineral elements in planed daily food ration (in mg)

	Na	K	Ca	P	Mg	Fe	Zn	Cu	Mn
Vegetables	566.2	1238.4	175.6	204.5	89.9	3.76	2.32	0.46	0.21
Fruits	8.5	783.9	69.4	68.5	58.5	1.36	0.48	0.35	0.53
Norm (at the safe level)	625	3500	1100	800	350	11.0	14.0	2.0-2.5	5.0

Conclusions

1. Vegetables intake with the daily food ration planned for soldier's alimentation in winter season is lower by 25% than recommended in the "Proper nutrition pyramid" amounts.
2. Planned in rations amounts of fruits exceeded minimum recommended by the WHO and specified as 300 g daily.
3. Sodium content in vegetables planned in soldiers' alimentation met 90% of the requirements obligatory in Poland.

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Bioavailability of magnesium ions in aspect supplementation

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INTRODUCTION

Magnesium ion is one of the most important cation in an organism. From our previously examination we concluded that in many cases hypermagnesaemia is possible status. Bioavailability magnesium from pharmaceutical dosage form as the tablets is good and our analysis daily menus indicated that deficit magnesium in diet was not often (2,3).

Fatty acids in cell are well know as a sources of energy and component building structures of cell (1). The process of theirs estrification and oxidation is key in the way of metabolic transformation. We sought determine changes in cells as isolated rats hepatocytes, induced high concentration magnesium ions and we compared these changes with these ethanol induced. One should ethanol to causing factors first of all process peroxidation of fatty acids, formations of free radicals and what it binds changes in profil of fatty acids of the cells.

MATERIAL AND METHODS

Material to investigations were isolated rats hepatocytes. The process of isolation was provided according to Seglen method (4). Viability of the cells ranged between 85-98% assessed by trypan blue exclusion. Next, the hepatocytes in amount $1,3 \times 10^7$ cells were plated in 10 cm^3 Nunclon flasks in Hepatocyte Medium. To the medium we added respectively: A and B MgCl_2 , C - $80 \mu\text{L}$ of ethanol, D and E - $\text{MgCl}_2 + 80 \mu\text{L}$ of ethanol. The final concentration MgCl_2 in the sample A,B,D,E was 2 mM and in B 4mM.

The control samples were hpatocytes incubated in the medium without any additions and treated in the same conditions. All operations connected with the isolation and preparation of medium were carried out in aseptic conditions in a laminar chamber. The samples were incubated at the temperature of 37° degree for 5 hours in a chamber – Heraus Instruments BB16, in atmosphere of 5% CO_2 . After this period the hepatocytes were centrifuged 50xg 10min. and supernatant was removed and the hepatocytes were washed 3 times with physiological saline. Disintegration of the cells was moved by repeated freezing and defrosting in liquid nitrogen. Next, we obtained after centrifugation the membranes of hepatocytes. The samples were homogenized in mechanical homogenizator type Ultra-Turrex T8 and after liofilizated –liofilizator Heto FD25. The next step of experiments was qualitative and quantitative analysis of fatty acids in obtained samples according to Wollenberg and Rietschel (5). The methyl esters of fatty acids were identified by GC-MS system included the Hewelett-Packard 5980 mass spectrometer, Hewlett-Packard 5980 gas chromatograph. For statistical analysis we used Chi-square test. Value of $p < 0,05$ was accepted as the level of statistical significance. We identified fatty acids as methyl esters on

the basis of retention times and comparison with that from libraries. The quantitative analysis based on area under the peaks – AUPs which answers suitable fatty acids.

RESULTS

Tab1. Quantitative changes in the pool of total fatty acids in hepatocytes membranes after 5h incubation with magnesium or/and ethanol (normalized AUPs)

Fatty acid	4MgCl ₂ +ethanol	2MgCl ₂ +ethanol	Ethanol	4mM MgCl ₂	2mM MgCl ₂	Control
C14:0	7	6	6	5	4	7
C15:0	2	2	2	3	2	2
C16:1	48	49	49	63	47	48
C16:0	193	172	179	146	191	179
C17:1	2	1	1	3	2	2
C17:0	3	3	3	4	3	4
Ci18:0	0	1	1	1	1	1
C 18:2	53	63	70	62	44	66
C18:1a	115	112	110	144	111	106
C18:1b	34	32	33	40	44	33
C18:0	145	136	140	128	150	146
C20:4b	0	2	5	4	0	2
C20:3b	0	0	1	0	0	0
C22:6b	0	19	5	5	6	4
C22:0	2	2	2	0	2	2
C24:0	5	9	2	1	2	7

Tab. 2. Quantitative changes in the pool of ester-bound fatty acids in hepatocytes membranes after 5h incubation with magnesium or/and ethanol(normalized AUPs)

Fatty acid	4MgCl ₂ +ethanol	2MgCl ₂ +ethanol	Ethanol	4MgCl ₂	2MgCl ₂	Control
C14:0	2	2	2	3	2	2
C15:0	1	1	1	1	1	1
C16:1	21	20	23	17	21	18
C16:0	66	58	57	65	58	61
C17:1	2	0	1	1	1	0
C17:0	0	2	1	1	1	1
Ci18:0	0	1	2	0	1	0
C 18:2	30	31	21	20	27	22
C18:1a	49	46	54	40	50	46
C18:1b	0	13	13	11	14	14
C18:0	42	40	42	54	41	48
C20:4b	1	1	1	1	1	0
C20:3b	0	1	0	0	0	0
C22:6b	4	2	0	1	1	5
C22:0	0	0	0	1	0	0
C24:0	1	1	1	3	0	1

CONCLUSIONS

The amount of total fatty acids in the hepatocytes membranes changed after 5h incubation with magnesium ions or/ and ethanol.

The similar tendence was observed when the ester-bound fatty acids were taken into consideration.

LITERATURE

The literature is available with the author.

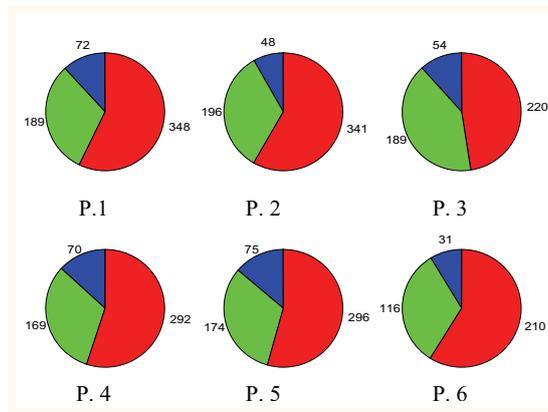


Fig.1. The amount of saturated [right segment], mono- and polyunsaturated fatty acids in membranes of hepatocytes after incubation P1-control, P2- with 2mM MgCl₂, P3-with 4mM MgCl₂, P4- with ethanol, P5-with ethanol and 2mM MgCl₂, P6-with ethanol and 4mM MgCl₂.

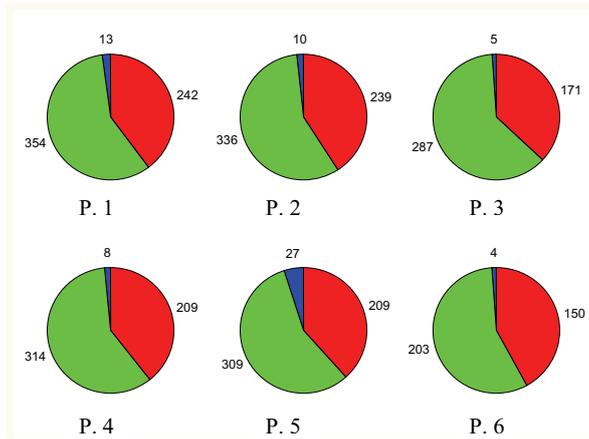


Fig.2. The amount of C14-C17 [right segment], C18-C20 and C21-C24 fatty acids in membranes of hepatocytes after incubation P1-control, P2- with 2mM MgCl₂, P3-with 4mM MgCl₂, P4- with ethanol, P5-with ethanol and 2mM MgCl₂, P6-with ethanol and 4mM MgCl₂

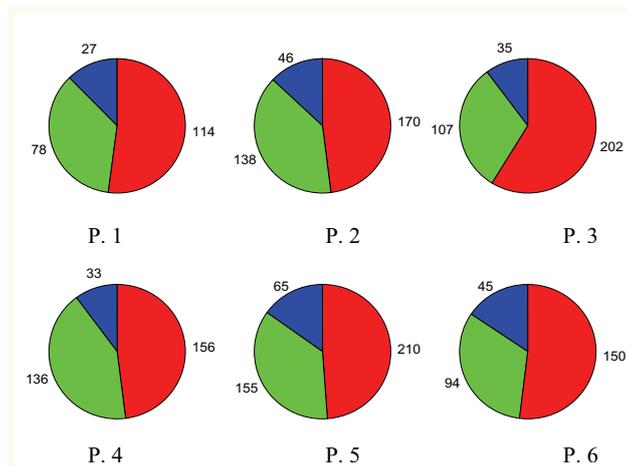


Fig. 3. The amount of **saturated** [right segment], **mono-** and **polyunsaturated** ester-bound fatty acids in membranes of hepatocytes after incubation P1-control, P2- with 2mM MgCl₂, P3-with 4mM MgCl₂, P4- with ethanol, P5-with ethanol and 2mM MgCl₂, P6-with ethanol and 4mM MgCl₂.

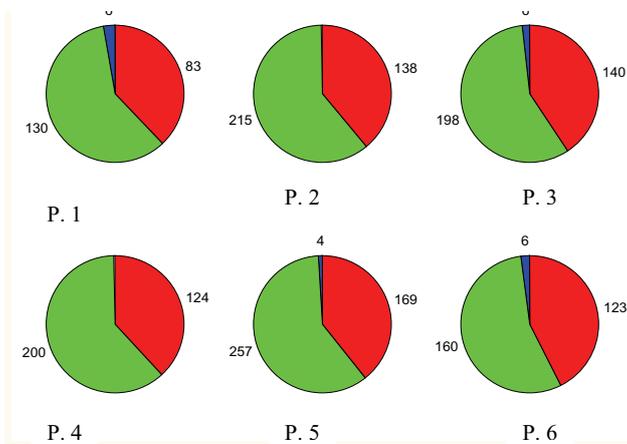


Fig. 4. The amount of **C14-C17** [right segment], **C18-C20** and **C21-C24** ester-bound fatty acids in membranes of hepatocytes after incubation P1-control, P2- with 2mM MgCl₂, P3-with 4mM MgCl₂, P4- with ethanol, P5-with ethanol and 2mM MgCl₂, P6-with ethanol and 4mM MgCl₂.

Iodized tea influence in childhood

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Introduction

Georgia is one among all those countries who somehow face the problem of iodine deficiency. The soil and water in the region are low in iodine due to the geography. Georgia lies in South of the European territory. The Caucasus, of which Georgia is apart, forms as isthmus that connects Europe to Asia. Georgia is mountains country and thus, iodine deficiency disorders always were characterized for this regions. This high mountain regions were known as endemic goiter hearth. One of the region lies next to the Black Sea. There are no territories in Georgia where the population is not under the risk of developing iodine deficiency disorders. Development of goiter in the seaside regions of the country is suggested to be possibly influenced by the Chernobyl incident. Despite efforts in the sub-region iodine deficiency is still a major health problem. In Georgia IDD have always been an endemic disease and this problem is still actual especially in high mountain regions. All above-mentioned made important to find alternative sources of iodization of food products in Georgia. The most effective method of the fight against iodine deficiency is iodine prophylactic, based on providing population of iodine-deficit regions with iodized product (salt, bread, milk, tea). It is established that day and night consuming of iodine products containing 100mcg of iodine, decreases the diseases of thyroid gland by 50-60% during a year. Iodine Deficiency Disorders (IDD) are one of the most widespread non-infectious diseases. 1.5 billion world population is under the risk of inadequate consumption of iodine; almost 655 million have enlarged thyroid gland (endemic goiter), while 43 million – mental retardation due to iodine deficiency. Over the last years studies revealed that iodine deficiency is not limited only to endemic goiter. Iodine deficiency causes relatively severe effect on the brain of young children, since induces development of unreversible changes. Even the modest deficiency of iodine can result in reduction of the intellectual potential of the population by 10% that represents a serious threat to intellectual and economic development of the nation. In 1997-2000 several epidemiological studies by endocrinologist on the National Center

of Nutrition and foreign specialists showed that in different regions of Georgia the prevalence of goiter spreading was 32% 48% in adults and between 29-44% in children.

Material and methods

Iodine is a crucial microelement for the human body. Iodine is essential in every age however its deficiency is particularly dangerous for children in early ages, since every organ and tissue, brain, skeleton, as well as intellectual and physiological potential is formulated from embryo to 3 years of age with participation of the micronutrient. If the iodine deficiency is not filled, subsequently the functional activity of the thyroid gland decreases, blood thyroid hormone levels drop metabolism slows down – hypothyroidism is developed. Thyroid hormone deficiency in fetus and newborns induces even more serious consequences – impairment of CNS development and formation of mental retardation. The level of mental retardation varies from mild (sub – clinical) to evident cretinism. Iodine deficiency results not only brain damage but also visual, auditory and articulation impairments. Children groups up mentally and physically retarded. Persons affected by IDD have lower learning capacities, undertake less complicated tasks; their work is less productive. As noted above, majority of Georgian regions have IDD at various levels iodine consumption by the population in Georgia on average comprise 40-80mcg/day. The following requirements developed for the adequate development of children and normal functioning of the adult organisms: 1. 90 mcg for children in early ages (2-6 years); 2. 120mcg for school age children (7-12 years); 3. 150mcg for adults (over 12 years); 4. 200mcg for pregnant and lactating women. For this reason prevention should carry a mass nature. The most effective method of the feight against iodine deficiency is iodine prophylactic, based on providing population of iodine deficit regions with iodated product (salt, bread, milk, butter, sugar, tea etc). In many countries iodated salt is preferred as a cheapest and effective measure. Moreover, salt is the only mineral, used by age groups of population. Apart of salt, in all population levels tea is an important component of their daily ration. Compared with salt, iodine tea is not transferred to the free iodine condition and is not evaporated as a result, tea reiodization is not needed. As Georgia traditionally is so called „tea country” exactly this product was chosen for this purpose. KJ was added in tea by original technology proper amount

with innovative way, using special mixer-dosimeter. Tea iodizing technology and method makes precise dozing possible, beginning from several micrograms and more (20, 30 ... 50mcg. etc). Having several tea – cups (2-3) we control the iodine day doze, because one packet of the tea for one cup contains 50mcg of iodine, that is 100 – 150mcg a day. During tea iodizing the taste, color and look of the product does not change. We have examined 228 children which leaved in the same social contamination.

Results

Except of iodized tea they become no another iodized products. Before giving them iodized tea this children were examined. Were collected urine samples for urine iodine excretion determination and examined thyroid gland by sonography. Prevalence of goiter spreading was 39% in one group it was 158 children and other 70 child had no goiter. Median of iodure in I group was 7,1mcg% and 9,3mcg% in the II group. The iodized tea was given twice a day. So the children became 100mcg iodine per day. The children from the first group we have divided in two parts. 33% of them had goiter of the I degree and 6% had goiter of the II degree.

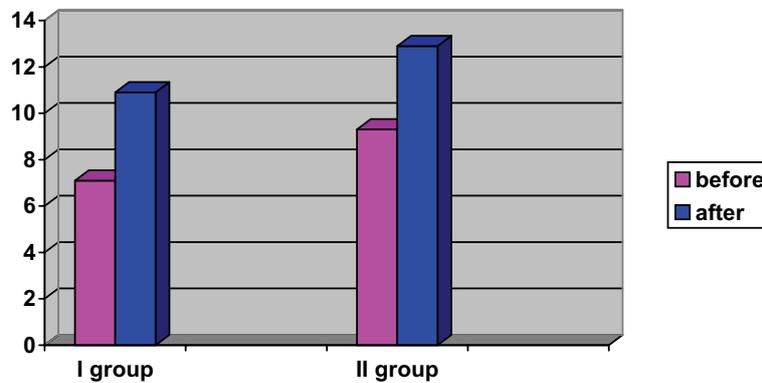


Figure 1. Mediane of iodine excretion in urine before giving iodized tea and after

After 6 month we became another figures. Frequency of goiter spreading in first group was decreased from 33% to 16%. In another group 2 children had no changes, but 7 of them had improvement from II degree goiter decreased to I degree. Median of iodine excretion in urine also changed. In I group it was 7.1mcg% and after 6 months it was 10.9mcg% ($P<0.05$), in the II group we had 9.3mcg% and became 12.9mcg% ($P<0.05$).

Summary

As we see use of iodized tea had positive results on children. It can be used not only in children, but also in adults, pregnant women and persons of different ages. Of course only iodized tea is not enough to solve IDD problem. But joint efforts of health institutions of Government and education of our population together will decide this problem. Iodization of other products in our country would help us to eliminate this problem in future.

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Influence of iodine and selenium supplementation to the diet of ewes (Black-head dairy breed) on the milk composition and trace element content of milk

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Introduction

The established imbalance of the basic essential elements (Se, I, Zn, Co) in the flora of mountain areas can affect negatively the milk production (Makaveeva et al., 2004; Petrova et al. 2002). The large population size of different sheep breeds, their wide adaptability to climates and forage quality, make sheep widely accepted and genetically of greater potential. Many authors have proved that adding of inorganic components are important for the ewes milk yield and milk quality, daily fat and protein secretion (Odjakova 2000). Selenium deficiency led to decreased milk production and a reduction of the daily milk fat and protein secretion. The objective of the study was to establish the effect of Se and I supplementation to the basal diet of ewes on the milk production, daily fat and protein secretion, trace elements (Se, I and Zn) concentration in the milk during the first 56th days of lactation.

Material and Methods

The experiment was carried out with 20 ewes of Blackhead dairy breed, divided into two groups. The basal diet included 0.08 mg Se/kg DM and 0.10 mg I /kg DM. The control group received additionally *per os* 2.10 mg Se as NaHSeO₃ and 2.8 mg I as KI every two weeks. Milk samples were taken on the 14th, 28th, 42nd and 56th day of lactation. Milk protein was determined using Milk tester of Foss Electric (Denmark) and milk fat by Gerber's method. The Se-content was analyzed by means of AAS-HS. The I-analyses were carried out according to the Sandell-Kolthoff-method. Zinc was determined with Flame-AAS. Statistical analyses were performed using General Statistic Pack of Hewlett Packard.

Results

The average daily milk production (813 ml/d) of control group demonstrated the biological effect of trace element supplementation (+Se +I) in the ration of Blackhead sheep in comparison with the Se-I-deficient group (674 ml/d). Supplemented ewes produced daily 139 ml more milk with natural fat content (20,6%). The same results were found in experiment with Blackhead x Avassi (Angelow et al., 2000).

The obtained results confirmed the tendency that Se-I unsupplemented animals produced significantly less milk (-17%), but the effect was lower compared to results for the South Corriadale breed - 30% (Makaveeva et al. 2004).

Table 1. Influence of iodine and selenium supplementation on the average milk production and milk composition during the lactation period (14-56 day)

Parameter (n= 40, 40)	Supplemented group	Deficient group	p	%
milk (ml/day)	813 ± 38	674 ± 76	< 0.001	83
fat content (%)	5.11 ± 0.78	6.19 ± 0.46	< 0.001	121
protein content (%)	4.94 ± 0.08	4.76 ± 0.23	< 0.05	96
fat secretion (g/day)	41.56 ± 6.46	41.90 ± 7.8	> 0.05	101
protein secretion (g/day)	39.83 ± 2.17	31.97 ± 2.1	< 0.001	80

The multi-element deficiency influenced also the fat contents and the daily protein secretion (Tab. 1). The deficient ewes produced milk with higher fat content. Se-I-deficient group produced 20% less milk protein daily ($p < 0.001$). The percentage of fat in ewe's milk increased in the Se-I deficiency group (6.19% vs. 5.11%). The effect of balanced nutrition on the Se, I and Zn concentration in milk and the daily trace element secretion was clearly demonstrated during the whole investigated period (Fig. 1,2,3 and Tab. 2,3,4) .

> Selenium

The addition of selenium and iodine influenced significantly the concentration of Se in the ewes milk (Fig. 1).

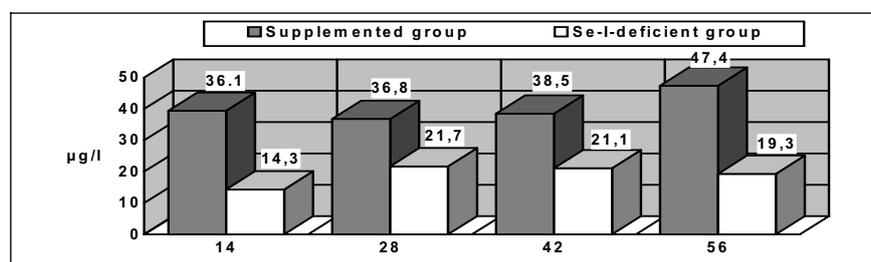


Figure 1. The influence of iodine and selenium supplementation on selenium content of milk during the first 56. day of lactation

The supplemented ewes secreted on the average twice as much Se into milk than the deficient animals (39.7 µg Se/l vs. 19.1 µg Se/l). These results correlated very well with the findings of many authors (Petrova, 1998). The low selenium content in the ration influenced high significantly the daily Se-secretion (µg/d) into milk (Tab. 2). The supplemented group secreted on the average 2,52 fold more Se into ewe's milk in comparison with the deficient ewes (32.2 ± 3.3 vs. $12.8 \pm 1,6$ µg Se/day).

Table 2. Daily selenium secretion during the lactation period ($\mu\text{g}/\text{day}$)

day of lactation	14	28	42	56
supplemented group*	28.9 ± 2.6	31.8 ± 3.1	31.3 ± 2.4	36.8 ± 1.5
I-Se-deficient group**	11.2 ± 1.8	14.5 ± 1.6	13.7 ± 1.4	11.6 ± 1.2
p	< 0.001	< 0.001	< 0.001	< 0.001
%	258	219	228	317

*supplemented group = X%; **deficient group = 100%

> Iodine

The deficient nutrition had also a negative effect on the I-transfer in the milk (Fig. 2).

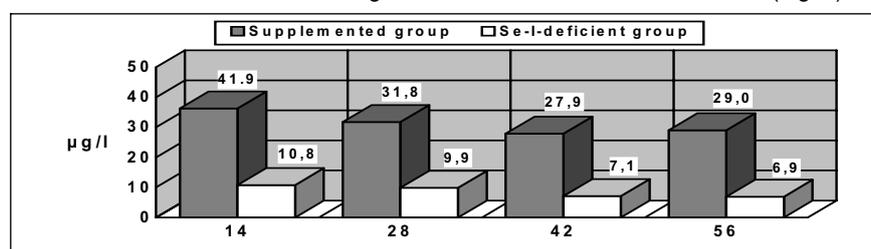


Figure 2: The influence of iodine and selenium supplementation on iodine content of milk during the first 56th day of lactation

The I-milk content of supplemented ewes remained almost constant at all measured points. In the deficient group the I-concentration decreased continuously from 10,8 $\mu\text{g}/\text{l}$ to 6,9 $\mu\text{g}/\text{l}$ with the advancing of lactation. In this case an additional decrease of I-content in milk was observed, because under Se-I-deficiency conditions an increase of the antagonistic effect between both elements exists. The difference in the average I-concentration in milk between the two groups was significant ($32,7 \pm 6,4 \mu\text{g l/l}$ vs. $8,6 \pm 1,9 \mu\text{g l/l}$).

Table 3. Daily iodine secretion during the lactation period ($\mu\text{g}/\text{day}$)

Day of lactation	14	28	42	56
supplemented group*	$33.5 \pm 5,3$	$27.5 \pm 4,2$	$22.7 \pm 2,5$	$22.5 \pm 1,8$
I-Se-deficient group**	$8.4 \pm 1,6$	$6.6 \pm 1,2$	$4.6 \pm 0,9$	$4.1 \pm 0,7$
p	< 0.001	< 0.001	< 0.001	< 0.001
%	399	417	492	549

*supplemented group = X%; **deficient group = 100%

The daily iodine secretion in milk differed significantly (suppl. group 26.6 ± 5.2 vs. deficient group $5.9 \pm 2.0 \mu\text{g}/\text{day}$). During the whole period the supplemented ewes produced 4,5 times more iodine than the deficient animals.

> Zinc

It was found a smaller effect of the Se-I supplementation on the Zn content in ewes milk (Fig. 3).

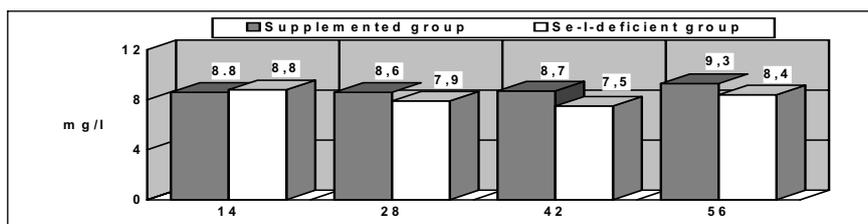


Figure 3. The influence of iodine and selenium supplementation on zinc content of milk during the first 56th days of lactation

The Zn-concentration in milk of supplemented group was higher after the 28th day. Differences in the Zn content of milk were not so clear as the obtained for selenium and iodine content.

Table 4. Daily zinc secretion during the lactation period (mg/day)

Day of lactation	14	28	42	56
supplemented group [*]	6.9 ± 0.5	7.4 ± 0.4	7.1 ± 0.8	7.2 ± 0.3
I-Se-deficient group ^{**}	6.9 ± 1.2	6.6 ± 0.8	5.1 ± 0.6	5.0 ± 0.5
p	> 0.05	< 0.05	< 0.001	< 0.001
%	100	113	139	145

^{*}supplemented group = X%; ^{**}deficient group = 100%

The daily Zn secretion in both groups varied from 13 to 45%. The supplemented animals secreted on the average 22% more Zn (7,2±0.2 vs. 5,9±1,0 mg Zn/day) into milk in comparison to the deficient one (p<0,01).

Summary

The low levels of Se and I in the ration led to a decrease in the daily milk production by 17%. Se and I amounts in the supplemented group were 2,5 and 4,5 times more. The effect of Se-I deficiency on the Zn content was smaller. The obtained results demonstrated the necessity of Se - I supplementation to the ration of lactating sheep.

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Effect of natural magnesium additives on the rumen fermentation *in vitro*

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Introduction

Most naturally occurring mineral deficiencies in herbivores are associated with specific regions and are directly related to the soil characteristics. Natural mineral additives, e.g. dolomite, silicate minerals (Váradyová et al., 2003a; Váradyová et al., 2003b), and caustic calcinated magnesite are used in the agricultural practice. Caustic calcinated magnesite serves as natural sources of magnesium in the form of MgO. However, no investigation of the impact of caustic calcinated magnesite on the rumen fermentation pattern and rumen protozoa has been carried out. Finally, the production of volatile fatty acids and gases by rumen microbes is generally influenced by macro-minerals (Durand and Komisarczuk, 1988; Komisarczuk-Bony and Durand, 1991). The aim of this study was to examine the influence of two types of caustic calcinated magnesite (Caustic magnesite CCM 86 and Agromag 78, Slovak magnesite works, Jelšava Inc.) sources on the rumen fermentation patterns with different substrates (meadow hay, wheat straw, cellulose amorphous, barley) and protozoan population in sheep *in vitro*.

Material and methods

Rumen fluid was collected three hours after the morning feeding from three rumen fistulated Slovak Merino sheep. Inoculum (rumen fluid and buffer) was pumped by an automatic pump into the preheated fermentation bottles containing one kind of substrate. The incubations were performed in the incubator for 72 h at 39 ± 0.5 °C. The concentration of ciliate protozoa was counted (after 24 h) microscopically according to the procedure described by Coleman (1978). Protozoa were identified according to Dogiel (1927) and Ogimoto and Imai (1981). The following four substrates (0.25 g / bottle) were used: meadow hay (MH), wheat straw (WS), amorphous cellulose (AC) and barley grain (BG). Meadow hay, wheat straw and barley grain were ground, sieved (particle size of 0.15 to 0.4 mm) bulked and stored in sealed plastic containers until required. Two magnesium additives Caustic magnesite (CM) and Agromag (AG)

(both from the Slovak magnesite works, Jelšava Inc.) in the amounts of 0.01 g were added into the culture bottles containing substrates. Chemical composition of the tested caustic calcinated magnesite is presented in Table 1. The fermentation process and metering system of gases and VFA was performed according Váradyová et al. (1998).

Table 1. Chemical composition of the tested caustic calcinated magnesite:

	MgO (%)	CaO (%)	Fe ₂ O ₃ (%)	SiO ₂ (%)	Al ₂ O ₃ (%)
Caustic magnesite CCM 86 (0-0.125 mm)	86.0	2.5	7.5	0.5	0.2
Agromag 78 (0-0.5 mm)	78.0	2.8	6.8	0.8	0.3

Result

Compared to the controls (Tables 2 and 3), lower values were obtained for total gas and methane production of MH, WS, AC and BG with both CM and AG additives. Lower values as compared to the controls were obtained in the total VFA production of MH, WS and BG with CM and AG. Compared to the controls, total VFA production for AC with AG was higher. The higher values of acetate production for MH, WS, CA and BG with CM and (or) AG were accompanied with the lower values of the mol percent of propionate. Only the mol percent values for acetate and propionate of MH and AC were not influenced by AG additive. The mol percent values for n-butyrate of WS, AC and BG with CM and n-valerate of AC with CM and WS with CM and AG were higher as compared to the controls. The type of tested substrates had a significant effect on the growth of individual ciliate species. The BG substrate significantly increased the cell concentration of *Entodinium* species, *Dasytricha ruminantium*, *Iso-tricha* spp., *Eremoplastron dilobum*, and *Ophryoscolex c. tricoronatus*. During fermentation of MH with AG the total protozoan concentration and the number of *Entodinium* spp. was significantly decreased.

Table 2. Fermentation parameters of MH and WS in vitro for 72 h

	Meadow hay (MH)			Wheat straw (WH)			SEM
	Contr.	CM	AG	Contr.	CM	AG	
Total gas	45.4	38.8***	39.6***	40.0	37.3*	38.8	0.6
Methane	4.5	1.9***	1.7***	5.1	1.8***	1.8***	0.2
Total VFA	65.8	61.9*	62.7 ^c	64.3	60.8*	60.6*	0.8
Acetate	68.1	70.9***	67.8 ^c	65.4	69.5***	69.0***	0.2
Propionate	18.8	16.8***	19.1 ^c	21.9	17.0***	18.6*** ^c	0.2
<i>n</i> -Butyrate	9.0	8.4	8.4	9.2	10.4**	8.7 ^c	0.2
<i>Iso</i> -Butyrate	1.0	1.0	1.0	1.0	1.1	0.9 ^c	0.1
<i>n</i> -Valerate	1.2	1.3	1.2	1.0	1.3***	1.2***	0.1
<i>Iso</i> -Valerate	1.7	1.6	1.7	1.4	1.5	1.3 ^c	0.03
<i>n</i> -Caproate	0.3	0.3	0.2	0.2	0.2	0.3	0.1
A:P	3.6	4.3	3.6	3.0	4.0	3.7	0.3
2H-recovery	41.0	33.2***	36.4*** ^b	53.9	34.0***	35.3***	0.5

^{a,b} P<0.01, 0.001 significant differences between CM and AG

*, **, *** P<0.05, 0.01, 0.001 significant differences from the controls

Table 3. Fermentation parameters of AC and BG in vitro for 72 h

	Amorphous cellulose (AC)			Barley grain (BG)			SEM
	Contr.	CM	AG	Contr.	CM	AG	
Total gas	62.1	59.2***	60.0**	50.2	48.4*	48.0**	0.4
Methane	4.0	1.9***	1.9***	5.0	2.1***	2.1***	0.3
Total VFA	66.4	62.1**	71.6*** ^c	66.0	60.0***	61.6***	0.5
Acetate	63.8	68.8***	65.1	60.9	69.6***	64.9*** ^c	0.5
Propionate	30.0	25.7**	30.0 ^b	26.8	24.5**	22.7***	0.4
<i>n</i> -Butyrate	6.3	11.3***	5.9 ^c	9.1	10.4*	8.6 ^c	0.3
<i>Iso</i> -Butyrate	1.1	1.0	0.9	1.2	1.0	1.0	0.1
<i>n</i> -Valerate	0.8	1.5***	0.8 ^c	2.0	1.9	1.8	0.2
<i>Iso</i> -Valerate	1.4	1.6	1.4	1.6	1.5	1.5	0.1
<i>n</i> -Caproate	0.1	0.3	0.01	0.3	0.2	0.1	0.1
A:P	2.0	2.4	2.0	2.3	2.8	2.9	0.2
2H-recovery	50.1	41.3***	44.4*** ^c	52.7	40.3***	40.9***	0.6

^{a,b} P<0.01, 0.001 significant differences between CM and AG

*, **, *** P<0.05, 0.01, 0.001 significant differences from the controls

The fermentation of AC with AG decreased the total protozoan concentration and the number of *Entodinium* spp., *Dasytricha ruminantium* and *Isotricha* spp. The fermentation of AC with CM decreased the number of *Isotricha* spp. compared to the control. Barley grain fermented with AG significantly increased the total protozoan concentration and the number of *Entodinium* spp. On the other hand, BG fermented with CM and AG decreased the number of large *Entodiniomorphids*.

Summary

From our *in vitro* results, we have concluded that total gas and methane production, total and individual volatile fatty acids were significantly influenced by both natural magnesium sources. From this point of view caustic calcinated magnesite can be principally use as promising additive to support the mineral metabolism and secondary to decrease methane production. The results and experiences from *in vitro* experiments can be useful for *in vivo* study and final results can lead to application in the agriculture practice.

Literature (please contact author)

Rare earth elements as alternative growth promoters for pigs

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Introduction

Rare earth elements (REE) include the 15 lanthanide elements lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium. Scandium, yttrium and thorium are commonly included with the REE because of their similar properties. Despite their name, the REE are in fact not especially rare. Each is more common in the earth's crust than silver, gold or platinum. The light REE (La through Eu) are more abundant than the heavy REE (Gd through Lu). In China, REE are used in agriculture as fertilizer in plant production and as growth promoter in animal nutrition already for some decades. Many Chinese studies showed partly beneficial growth promoting effects whereas the most spectacular is seen in poultry and pig production (Hu et al., 1999). Several feeding trials with REE were already carried out under western conditions (Rambeck and Wehr, 2005; Eisele 2003; Rambeck et al., 1999). So far mineral salt chlorides were used in most previous studies. In the present experiment we used REE-citrate, based on lanthanum, cerium, praseodymium and neodymium as dietary supplement. In addition to the investigations on energy and nitrogen metabolism in pigs (see manuscript Prause et al., "Der Einfluss Seltener Erden auf die Energie-, Kohlenstoff- und Stickstoffbilanz wachsender Ferkel"), the goal of the present study was to test the influence of REE-citrate as potential growth promoter for weaning piglets under Swiss feeding restrictions (focus on safe feed has priority rather than maximum growth rate). The present study was the basis for a diploma theses at ETH Zurich, Institute of Animal Sciences, Nutrition Biology (Caletka-Fritz, 2004).

Materials and methods

The feeding experiments (Table 1) were carried out under test station conditions with a total of 147 weaned

Table 1: Experimental design

mg per kg	Treatment		
	A	B	C
Citrate	100	-	-
REE-citrate	-	150	300

Large White piglets (30 days of age) during 5 weeks with 10 replications per treatment. The initial mean body weight (BW) amounted to 8.5 ± 1.5 kg and final BW was 21.4 ± 5.6 kg. The cereal based and steam pelleted feed was formulated to

Table 2: Diet composition in %

Barley, Wheat, Oats, Maize	65.0
Rolled Oats	10.0
Extr. Soybean meal	7.0
Patato Protein, Fish meal	7.5
Organic acids, Dextrose	2.6
Amino acids, Minerals	2.7
Animal fat	2.5
Vitamins, Trace elements	0.6
Celite	1.1
Variable supplements	1.0

contain all nutrients required by weaner feed (Table 2 & 3) as recommended and given in dry form

in automatic feeders (*ad libitum*). The experimental feed was produced according to a 2 step-procedure (1st basal diets, 2nd addition of the various supplements to the blended basal diets). The piglets were kept in groups of 5 animals in pens with an insulated laying area, dung area and artificial ventilation system with an average temperature between 20°C to 23°C and 45% to 60 % relative humidity. Body weight (individual) and feed intake (group) were recorded weekly, feces condition daily (visual, 3 point scheme). Feed digestibility, after feces collection on day 15 and 35, was calculated by using the indicator method.

Table 3: Nutrient and REE content of the experimental diets (analyzed)

		Treatment		
		A	B	C
Analyzed, per kg feed (88 % dry matter)				
Crude ash	g	56	55	57
Crude protein	g	179	178	181
Crude fat	g	53	53	56
Crude fibre	g	38	31	35
Gross energy	MJ	17.0	17.2	17.3
Lanthanum	mg	0.15	7.6	16.9
Cerium	mg	0.25	22.7	49.0
Praseodymium	mg	0.04	3.9	8.0
Neodymium	mg	0.12	0.70	1.27

Results and discussion

The observed overall performance data (380 g body weight gain per day and 1.66 kg kg^{-1} feed conversion ratio) of the piglets were on a level as expected. Health status of the piglets and feces conditions was not affected by REE-citrate (data not shown). Dry matter content of feces ranged between 29.9 and 30.6 % ($p=0.584$). Slight digestion irregularities, that occurred mainly between the first and second week of the

experiment were not treated by medicaments and disappeared at the latest during the second week of the experiment. Growth performance (Table 4), calculated over the whole period of 35 days after weaning, was not significantly influenced by dietary REE-citrate (treatment B and C) compared to the simple citrate (treatment A) supplementation and is in agreement with Eisele (2003) and Halle et al. (2003).

Table 4: Growth performance, energy and nitrogen digestibility

	Treatment			p-value
	A	B	C	
Daily body weight gain, g				
Days 1-14	245 ± 131	237 ± 102	227 ± 91	0.70
Days 15-35	418 ± 140	463 ± 137	466 ± 122	0.62
Days 1-35	387 ± 132	373 ± 120	370 ± 105	0.69
Average daily feed intake, g				
Days 1-14	392 ± 167	374 ± 124	365 ± 118	0.62
Days 15-35	810 ± 227	777 ± 208	746 ± 167	0.23
Days 1-35	643 ± 196	616 ± 170	593 ± 142	0.29
Feed conversion ratio, kg/kg				
Days 1-14	1.83 ± 0.72	1.69 ± 0.41	1.66 ± 0.22	0.88
Days 15-35	1.69 ^a ± 0.06	1.69 ^{ab} ± 0.10	1.62 ^b ± 0.12	0.04
Days 1-35	1.69 ± 0.12	1.68 ± 0.13	1.62 ± 0.12	0.15
Digestibility of energy				
1. Sampling period	0.825 ± 0.01	0.835 ± 0.01	0.823 ± 0.01	0.13
2. Sampling period	0.838 ± 0.01	0.839 ± 0.01	0.833 ± 0.01	0.47
Total	0.830 ^b ± 0.01	0.837 ^a ± 0.01	0.827 ^b ± 0.01	0.05
Digestibility of nitrogen				
1. Sampling period	0.791 ± 0.02	0.801 ± 0.02	0.796 ± 0.01	0.50
2. Sampling period	0.808 ± 0.02	0.807 ± 0.02	0.799 ± 0.02	0.47
Total	0.798 ± 0.01	0.803 ± 0.01	0.797 ± 0.01	0.44

Slight effects could be found during the first (days 1 – 14) and second period (days 15 – 35), but the variation of the data was except for feed efficiency (second period) too large for statistical significance. Similar results were obtained in a previous study by Rambeck et al. (1999) where REE-chloride did not influence performance data of piglets (7-18 kg BW). In contrast, Borger (2003) found significantly higher daily body weight gain and better feed conversion ratio in piglets from 18-35 kg BW. In a study with growing-finishing pigs significantly better performance data were described by

Kessler (2004) due to dietary REE-citrate. However, in the present study, only feed efficiency between days 15-35 was influenced by dietary REE supplementation. Energy and nitrogen digestibility was generally on a high level, and therefore hardly influenced by dietary treatments. Nevertheless, overall energy digestibility in treatment B was significantly higher than in treatment A and C and was consistent with findings by Prause et al. (2004) where energy digestibility was numerically higher due to REE supplementation ($p=0.06$).

Summary

The goal of the present study was to test the influence of REE-citrate as potential growth promoter for weaning piglets under Swiss feeding restrictions. The observed overall performance data of the piglets were very satisfactory and on a level as expected for the measured body weight range (8-20 kg). The digestibility coefficients were generally on a high level. The results indicate that growth promoting effects of REE under test station conditions were limited and not consistent with numerous other studies, but feed efficiency was positively influenced.

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Der Einfluss Seltener Erden auf die Kohlenstoff-, Stickstoff- und Energiebilanz wachsender Ferkel

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Einleitung

Zu den Seltenen Erden (SE) werden 17 Übergangsmetalle aus der dritten Nebengruppe des Periodensystems gezählt. Sie haben untereinander ähnliche chemische und physikalische Eigenschaften. Die vier häufigsten Vertreter sind Lanthan, Cer, Praseodym und Neodym. Ihr Hauptvorkommen liegt in China, wo sie industriell abgebaut, aufbereitet und seit über vierzig Jahren in der Landwirtschaft eingesetzt werden. Dort wurde in zahlreichen Studien über leistungsfördernde Wirkungen von SE bei fast allen Nutztierarten berichtet. Auch in der Ferkelmast sind durch SE zum Teil beträchtliche Leistungssteigerungen erreicht worden (Hu et al., 1999). Da aber sowohl die Haltungs- und Fütterungsbedingungen, als auch das genetische Potential der Tiere oftmals nicht mit den westlichen Bedingungen übereinstimmen, sind diese Ergebnisse nicht auch zwangsläufig auf europäische Verhältnisse übertragbar. Aber seit einigen Jahren sind vor allem in Deutschland und der Schweiz Studien durchgeführt worden, die auf eine Wirksamkeit der SE als Leistungsförderer auch unter europäischen Mastbedingungen schliessen lassen können. Wirkmechanismus und Wirkansatzstellen der SE sind aber noch nicht vollständig geklärt. Deshalb sollte in dieser Studie neben einer Evaluierung der SE als Leistungsförderer, der Einfluss SE auf die Verdaulichkeit, die Energie- (BE), Stickstoff- (N) und Kohlenstoffbilanz (C) wachsender Ferkel ermittelt werden. Anhand einiger Blutparameter und Mineralstoffgehaltsanalysen der Metatarsalknochen sollten weitere mögliche Einflüsse chronischer SE Gabe untersucht werden.

Material und Methoden

Es wurden 40 männliche, kastrierte Absetzferkel mit einem Anfangsgewicht von $8,57 \pm 0,8$ kg im Alter von 32.5 ± 5.5 Tagen in den institutseigenen Stallungen der ETH-Zürich aufgestellt. Alle zwei Wochen wurden vier Tiere paarweise jeweils zwei Futtermitteln zugeteilt. Das Futter wurde nach einer praxisüblichen Ferkelfutterrezeptur mit den Hauptkomponenten Mais, Gerste, Weizen, Hafer und Sojaschrot und einen Vitamin/ Mineralstoff- Premix gefertigt. Der Energiegehalt betrug pro kg Futter 14,5 MJ VES und der Proteingehalt 185 g/ kg Futter. Es wurde jeweils Celite als Indikator und als Versuchssubstanz entweder 150 mg SE- Citrat \approx (VG1) 300 mg Lancer[®] (Zehentmayer AG, CH-9305 Berg), 300 mg SE- Citrat (VG2) \approx 600 mg Lancer[®] oder 100 mg Na- Citrat als Kontrollgruppe (KO) beigemischt. Bis zu einem Gewicht von ca. 25 kg wurden die Tiere paarweise gehalten und danach, bis zur Schlachtung mit ca. 55 kg, einzeln eingestallt. Während der Mast wurden die Ferkel für jeweils 96 Stunden zweimal in Respirationskammern verbracht. In der ersten Phase, bei der die Tiere ca. 18 kg wogen, wurden die Ferkel paarweise in 1,21 m³ Volumen umfassenden Respirationskammern gehalten. Bei einem Ferkelgewicht von ca. 50 kg wurden die Tiere dann in einer zweiten Phase einzeln in Stoffwechselständen in 5,44 m³ grossen Kammern untergebracht. Die Messung wurde nach dem System der offenen indirekten Kalorimetrie durchgeführt (Hadorn, 1994). Während der Respiration konnte neben den Klimabedingungen der Sauerstoffverbrauch, die Kohlendioxid- und die Methanproduktion gemessen werden. Nach der Auswertung der Respirationdaten und Probenanalysen von Futter, Kot und Urin war es möglich, eine genaue C -, N - und BE - Bilanz zu erstellen und deren Verdaulichkeit zu berechnen. Die tägliche Zunahme (TZ), die Futtermittelaufnahme (FA) und die Futtermittelverwertung (FV) wurden mittels täglicher Futterrückwaage und mindestens wöchentlicher Waage der wachsenden Ferkel ermittelt. Von den Tieren wurde jeweils viermal Blut aus der Vena cava gewonnen und nach der Schlachtung der Metatarsus zu weiteren Untersuchungen entnommen.

Resultate und Diskussion

Bei der Auswertung der Mastleistungsparameter zeigten sich nur im Bereich der Futtermittelaufnahme bzw. -verwertung positive Ergebnisse, wobei nur die niedrigere Konzentration der Seltenen Erden signifikante Effekte zeigte. Die tägliche Zunahme war in allen gemessenen Wachstumsperioden zwischen den drei Futtermitteln nicht verschieden und betrug ca. 800 g täglich. Die Futtermittelaufnahme der Ferkel der KO war

während des Versuchszeitraumes 9 % höher als in VG1 und 5 % höher gegenüber VG2, jeweils nicht signifikant. Die FV der VG1 verbesserte sich gegenüber der KO um 7 % signifikant ($p < 0.05$). Die FV der VG2/ KO war nicht signifikant 2 % niedriger. Die Auswertung der Daten wurde für beide Respirationsphasen separat durchgeführt. Die folgenden Ergebnisse beziehen sich nur auf die zweite Respirationsphase. Deshalb gehen von allen Gruppen jeweils 12 Ferkel in die Bewertung ein ($n = 12$). Die BE-, N- und C- Aufnahme war, während der zweiten Respirationsphase, in allen Gruppen ungefähr gleich hoch. In der Stickstoffbilanz zeigt sich zwar kein signifikanter Unterschied zwischen den Varianten, aber tendenziell war der Proteinansatz gemessen an der metabolischen Lebendmasse ($LM^{3/4}$) der VG1/ KO um 16 % ($p = 0.104$) und die N- Retention/ N- Aufnahme um 13 % ($p = 0.054$) erhöht. Die Daten der VG2 weisen hier keine deutlichen Unterschiede zu den anderen Fütterungsvarianten auf. Sowohl in der BE-, als auch in der C- Bilanz ergaben sich keine signifikanten Unterschiede zwischen den Versuchsgruppen. Die Verdaulichkeit BE, C und N waren in der VG1 tendenziell jeweils um 1 bis 2 % gegenüber der KO erhöht, aber nicht signifikant. Die vorliegende Studie zeigt ähnlich wie Gebert et al., (2005), aber im Gegensatz zu Ergebnissen von Knebel (2004) und Kessler (2004) keinen Einfluss auf das Wachstum der Ferkel. Allerdings ist die Futtermittelverwertung der VG1/ KO deutlich verbessert. SE können *in vitro* je nach Konzentration hemmend oder stimulierend auf Bakterienwachstum wirken (Muroma, 1958). Experimente im künstlichen Pansen und Gastrointestinaltrakt von Ferkeln konnten bisher aber keinen sichtbaren Effekt SE auf die Mikroflora *in vivo* aufzeigen (Knebel, 2004; Kraatz, 2004). Daneben ist in Diskussion, ob SE die Permeabilität und den aktiven Nährstofftransport des Dünndarmes beeinflussen und somit zu einer erhöhten Absorption der verdaulichen Substanzen beitragen könnten. Auch ein Einfluss auf Enzyme des Verdauungstraktes und nach Absorption auf intra- und extrazelluläre Vorgänge des metabolischen Stoffwechsels ist möglich. Denn SE können durch ihre physikalische und chemische Ähnlichkeit mit Calcium²⁺- und anderen Alkalimetallionen, diese an spezifischen Bindungsstellen ersetzen und so einen stimulierenden oder je nach Konzentration auch hemmenden Einfluss auf zahlreiche Stoffwechselfvorgänge ausüben. (Evans, 1990; Arvela, 1979). Damit könnten sich dosisabhängig positive oder sogar negative Effekte auf Wachstum und/ oder Futtermittelverwertung ergeben.

Summary

The impact of rare earth elements on energy-, carbon-, and nitrogen balance of growing piglets

Daily body weight gain was not affected by supplementing REE citrate, as seen in a few other studies, too (Böhme et al., 2002). This is in contrast to results of Knebel (2004) and Kessler (2004). Feeding the low dose of REE significantly reduced feed conversion rate about 7% ($p < 0.05$), because daily feed intake was 9% lower as the control group. Piglets that were fed the high dose of REE showed a reduced feed intake of 5% and the conversion rate was improved by 2% to control but statistically not significant (ns). To Muroma (1958), REE are able to stimulate or inhibit bacteria dose dependent. But Knebel (2004) and Kraatz (2004) did not evaluate any effects of REE on gut flora and digestibility. REE are able to replace Ca^{2+} ions junctions and so they could have an effect on intestinal enzymes and after absorption could have an impact on intra- and extra cellular metabolism. This study presents, that an impact of REE is dose dependent. Fed in moderate doses, probably it could be an alternative growth promoter for growing piglets. Further studies should be conducted to evaluate REE as growth promoter, to detect more about the mode of action and to ascertain the optimal doses of REE in feed.

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Effects of the multi-element supplementation to the diet of ewes on the reproduction parameters and growth of female lambs reared in endemic regions of Middle Bulgaria

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Introduction

The extensive system of husbandry of small ruminants is associated with some nutrient imbalances that can occur due to the irregular mineral supply through natural pastures. Great variations exist in trace element contents of meadow vegetation in depends on different factors: plant species, stage of maturity, season, chemical properties of soil. The low availability of Zn, Se and J is typical for mountainous areas and corresponds to geological origin of pastures. The Zn-Se-J deficiency affects different paths of metabolism and animal production losses may exceed 20% (Kashamov et al., 2005; Petrova et al., 2000).

The aim of present study was to assess effects of the Zn-Se-J addition on certain reproductive parameters in ewes raised in an endemic region and the growth performance of female lambs.

Material and methods

Twenty five ewes of the South Corriedale breed at the fourth lactation were divided into the supplemented (n=13) and deficient (n=12) group. With the exception of the insufficient Zn-Se-J supply of non-treated group ewes were fed a diet adequate to nutrient requirements (AFRC, 1995), based on 0.5 kg grass hay, 0.5 kg alfalfa hay and 0.5 kg concentrates. The deficient ewes received Zn-35.8, Se-0.081 and J-0.060 mg kg⁻¹ DM. During pregnancy and suckling period the diet of supplemented ewes was added with 0.15 mg Se as NaHSeO₃, 0.15 mg J as KJ and 25 mg Zn as ZnSO₄·7H₂O per kg DM. After weaning the growth rate of the female lambs, correspondingly divided into supplemented (n=7) and deficient (n=7) group, has been controlled over a period of 180 days. The feeding regimen of lambs included a restricted diet composed of grass hay, alfalfa hay and concentrates in proportion 30: 30: 40 providing 110 g crude protein per kg dry matter. The ration of the deficient group contained Zn – 26.8, Se - 0.071 and J - 0.081 mg kg⁻¹ DM. The supplemented lambs received additionally 25 mg Zn as ZnSO₄·7H₂O, 0.25 mg Se as NaHSeO₃ and 0.25 mg J as KJ per kg dry matter.

During the experimental period the following parameters were estimated: trace element concentration in forages and animal tissues, mortality rate, body weight gain and feed utilization. The Zn concentration was measured by AAS, the Se levels - by HG - AAS and the J content by a colorimetric method according to Groppe et al. (1989). Results were expressed by mean \pm SD and compared by t-test criterion.

Results and discussion

According to diagnostic criteria shown on Table 1 it has been established that the insufficient supply in the region lead to a severe depletion of ewes and lambs organism.

Table 1. Effect of the insufficient Zn-Se-J exposure on the trace element status

Element/ tissue	Ewes 5-years-old	Lambs 3-months-old	Reference values for normal status
Zn/ liver mg kg^{-1} DM	173 \pm 26	158 \pm 24	200 ¹⁾
Se/ cardiac muscle $\mu\text{g kg}^{-1}$ DM	256 \pm 38	238 \pm 94	900-1000 ²⁾
J/ weight of thyroid gland g	35 – 42	17 - 32	15-17 ³⁾

¹⁾ McDowel et al. (1984); ²⁾ Anke et al. (1983); ³⁾ Groppe (1986)

The multiple deficiency was found to influence considerably the live expectance of offspring (Figure 1).

The prenatal mortality accounted to 9% in the deficient group was predetermined by the low intrauterine transfer and subsequently increased embryonic death.

The sparing effect of Se and J deficiency inducing degeneration of Striated muscle and cardiac muscle and impaired energy metabolism resulted in high mortality rate most pronounced during the first 7 days after parturition (38% neonatal mortality in the deficient group).

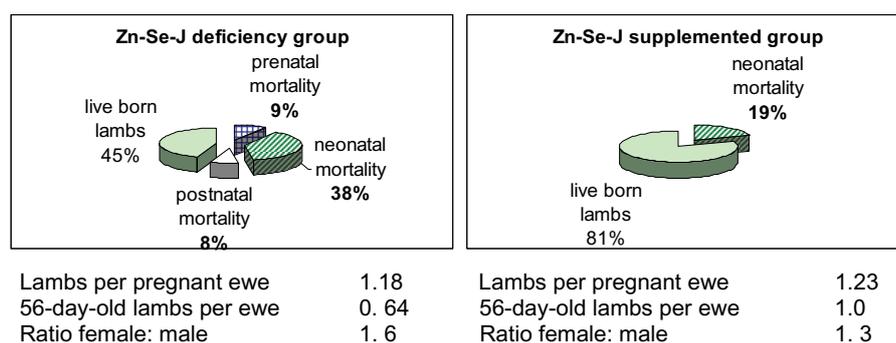


Figure 1. Effect of the Zn-Se-J supplementation on certain reproductive parameters

Lambs were found to lack vitality and more affected were the males, as previously reported (Petrova et al., 2000). The additional Zn-Se-J supply was established to have a substantial effect on mortality rate, expressed by the elimination of prenatal and postnatal mortality and by the reduction of neonatal mortality to 19%. The sex ratio was slightly changed to the higher number of male lambs survived.

The expected influence of supplementation during suckling period was not established. The difference in birth weight accounted only to 0.4 kg ($P > 0.05$) and both groups reached the same weight at weaning (11.71 ± 2.70 kg and 12.19 ± 1.94 kg in the deficient and supplemented group, respectively). The lack of effect may be accounted for the extremely lowered status of ewes after the prolonged deficient exposure. It was also associated with the very low milk performance (294 ml/day in the deficient group and 342 ml/day in the supplemented one).

During the subsequent period of 180 days after weaning a positive effect of the Zn-Se-J addition on growth rate has been demonstrated. Imbalanced nutrition and upset health status of the deficient group resulted in growth rate depression during the first 60 days (Figure 2).

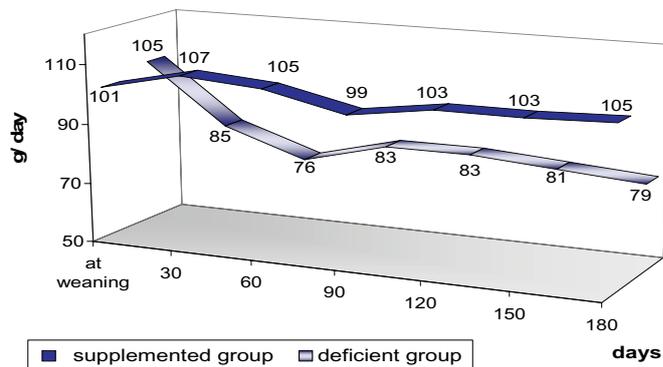


Figure 2. Effect of the Zn-Se-J supplementation on the average daily gain of female lambs of South Corriedale breed

During the whole period supplemented animals demonstrated significantly higher average daily gain ($P < 0.01$) at a constant rate due to the restricted diet. Data on Table 2 summarize the total effect of supplementation.

By the end of testing period supplemented animals had significantly higher body weight (31.17 ± 3.08 kg vs. 25.95 ± 4.18 kg, $P < 0.05$). They reached 4.75 kg more live weight gain ($P < 0.01$). Since there were no differences in feed intake the estab-

lished effect should be entirely related to the more effective feed utilization of the supplemented group expressed by the 33% lower feed consumption per kg gain.

Table 2. Effect of the Zn-Se-J supplementation on growth performance of female South Corriedale lambs (180 days after weaning)

Indices	Deficient group	Supplemented group	Difference *
Feed consumption, g/ day	992	992	-
Protein intake, g/ day	110	110	-
Starting body weight, kg	11.71 ± 2.70	12.19 ± 1.94	P > 0.05
Final body weight, kg	25.95 ± 4.18	31.17 ± 3.08	P < 0.05
Live weight gain, kg	14.24	18.99	P < 0.01
Intake: Gain, kg/ kg	12.6	9.74	77%

* Deficient group = 100%

Results obtained were in consistence with the previously reported data on the particular effects of elements and their synergistic interactions (Minson, 1990).

Summary

The experimental addition of 25 mg Zn ($ZnSO_4 \cdot 7H_2O$), 0.15 mg Se ($NaHSeO_3$) and 0.15 mg J (KJ) per kg to the diet of the Zn-Se-J deficient ewes during pregnancy was established to eliminate the prenatal and postnatal mortality of offspring and to decrease the neonatal mortality from 38% in non-treated group to 19% in the supplemented one.

Over a period of 180 days after weaning the effect of addition of 25 mg Zn ($ZnSO_4 \cdot 7H_2O$), 0.25 mg Se ($NaHSeO_3$) and 0.15 mg J (KJ) per kg to the diet on growth performance of the female lambs has also been tested. The upset health status of the deficient group was found to result in growth rate depression established during the first 60 days. By the end of experiment under conditions of restricted diet the supplemented lambs had higher live weight gain by 4.75 kg reached through more efficient feed utilization (intake: gain) by 33%.

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Organic acids in animal feeds – improving the decision making process

(*Organische Säuren in Futtermitteln - den Entscheidungsprozess verbessern*)

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The EU-wide ban on the use of in-feed sub-therapeutic levels of antibiotics has focussed interest on the role of organic acids in animal feeds. Apart from optimising feed hygiene there is also the potential for encouraging a favourable microflora in the digestive tract. There are a number of factors to be considered when developing a strategy for acid use: 1) Level of inclusion that will ensure a consistent response – in some cases the actual concentration and acid composition in commercial blends is not stated providing no information upon which to make decisions on the optimum inclusion rate. 2) Single acid or blend – blends of methionine hydroxy analogue (Alimet[®]) and formic acid for example, have been shown at acid pH *in vitro* to be significantly more effective against *Salmonella enteritidis* than the individual acids under the same conditions. 3) Use of free acid or salt – whereas for handling the use of acid salts has advantages data from *in vitro* experiments show that acid salts may be less effective at modifying the microbial population than inclusion of the free acid in feed. 4) Encapsulated or protected acids – this approach will only be cost effective if the protective matrix is truly acid resistant and also if the blend of acids released is active at the pH of the target intestinal site. For example, incubation of *S. enteritidis* at pH 7.0 (Initial Optical Density (OD) at 600nm of 1.06) with either sorbic or fumaric acids at 0.5% resulted in final ODs of 0.74 for formic acid and 0.22 for sorbic acid, indicating that sorbic acid was more effective than formic acid at this pH.

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The antimicrobial and acidification effects of ACTIVATE WD, an organic acid blend containing ALIMET® with other organic acids, in poultry drinking water

(Antimikrobielle und ansäuernde Effekte von ACTIVATE WD, einer Mischung organischer Säuren bestehend aus ALIMET® mit anderen organischen Säuren, im Tränkwasser für Geflügel)

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The antimicrobial property of DL-2-hydroxy-4-(methylthio) butanoic acid (HMTBA or Alimet®) has been previously documented (Hao and Schasteen, 1999; Enthoven 2002) in culture broth. We have also evaluated the effect of formic, fumaric, propionic, butyric, lactic, HMTBA and HMTBA-containing organic acid blends (Activate) on *Salmonella* growth in feed using a simulation of conditions (moisture and pH) encountered in the stomach and proximal to the small intestine. The addition of HMTBA to individual or a blend of organic acids resulted in a more than additive increase in antibacterial effect against *salmonella* in this assay. We have also done field testing of our HMTBA-containing organic acid blend for the drinking water, Activate WD. Three trials (1 22K broiler house treated and 1 untreated per trial) at different locations were conducted with Activate WD (0.04-0.08%, pH ~3) included in drinking water for the last 10-14 days prior to processing. Cloacal swabs of birds were positive for *salmonella* prior to starting Activate WD while swabs taken after treatment and prior to transporting the birds to the processing facility were found to be *salmonella* negative. This does not guarantee the chickens have no *salmonella* present in their intestine but indicates that they were not shedding. Live weight increases were seen in all 3 trials with an average increase of 140.7 grams/bird compared to non-treated houses. Activate WD treated birds water intake was not different than untreated controls. The presence of HMTBA-containing organic acid blends in the feed and or water provide antimicrobial and acidification benefits.

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Interest for utilizing a single multi-enzyme preparation on different types of diets from post-weaning piglets to slaughter pigs

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The interest to utilize a single multi-enzyme preparation (Rovabio™ Excel, Adisseo, France) in order to increase the nutritive value of swine diets was examined by means of different digestibility and performance trials. Different dietary regimes, namely wheat-, wheat/barley- or corn-based diets, as well as different physiological stages, from post-weaning piglets up to slaughter animals, were studied. For each variable two studies were conducted by changing the dietary compositions. In piglets, faecal digestibilities of Crude Energy and Crude Protein were determined using either total faecal collection or grab samples of faeces by means of determination of an indigestible marker. Digestibilities in growing pigs were determined on an ileal level using ileal-rectal anastomosed animals. Performance trials were performed with animals kept either individually or grouped in floor pens, according to classical performance trials. Data were analysed using the ANOVA or ANCOVA procedure of Statview.

The conducted trials proved that by the utilization of a single multi-enzyme preparation it is possible to increase energy digestibility (+ 2% in piglets fed corn- or barley-based diets; 4.4% and 2.8% for fattening pigs when fed diets based on barley or wheat, respectively). Moreover, animal performance was improved (6 – 7% in average daily gain for piglets; 3.6% and 4.6% for fatteners) independently of the cereal raw material used to formulate the experimental diets and the physiological stage of the animals. With a single multi-enzyme preparation it is therefore possible to increase the digestibility of different types of diets and therefore the animal performance. This effect can be observed for piglets as well as for growing-finishing pigs. For the nutritionist it is therefore possible to change feed raw materials or to formulate feeds for animals in different physiological stages without changing the enzyme preparation.

Effects of the phytogenic feed additive FRESTA[®] F Conc. in weaned piglets

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Introduction

A ban of antimicrobial growth promoters (AGP) will be in force within the EU in 2006. There are also regulations for reducing ammonia and other gas emissions in livestock breeding (IPPC regulation). Great efforts have been made to find alternative feed additives for keeping performance and health of farm animals at high levels and to reduce gaseous emissions from livestock. Phytogenic (plant based) feed additives are mixtures of herbs and spices or their extracts, which contain a variety of bioactive compounds, like essential oils or flavonoids. Essential oils can improve feed intake according to their aromatic properties. Essential oils also stimulate the production of digestive juices (Platel and Srinivasan, 2004) and thus, improve digestibility. These effects are most important in young, weaned animals, where gut function is not fully developed. There is also indication for immune stimulation and regulation of gut microflora by phytogenic substances. The objective of the present study was to investigate the effects of the phytogenic feed additive Fresta[®] F Conc. (FFC) in weaned piglets in comparison and/ or in combination with AGP.

Material and Methods

A total of 96 piglets, averaging 5.5 ± 0.11 kg in body weight were assigned to four treatment groups according to body weight. Piglets were housed in groups of four

animals and fed on a control diet (NC; negative control: no supplements) or the control diet supplemented with a mixture of in-feed antibiotics (PC; positive control: chlorotetracycline, sulfathiazole, penicillin at 0.1% in diet) or Fresta[®] F Conc (NCF; at 0.03% in diet) or AGP (0.1% in diet) plus FFC (0.02% in diet). Corn - soybean meal based diets were formulated to contain 14 MJ ME per kg feed and 24% protein (day 1-14) and 22% protein (day 15-28) and 20% protein per kg feed (day 29-49), respectively. Pigs had *ad libitum* access to feed and water. Chromium oxide was added at 0.2% to the diets as indigestible marker for calculation of nutrient digestibility. Feed and faeces samples were analysed for dry matter and nitrogen concentration according to AOAC methods. Chromium oxide was determined by UV spectrophotometry (Shimadzu, UV-1201, Japan). Blood samples were taken via jugular vein from ten pigs of each treatment (day 1 and 49) and were analysed for the concentration of red (RBC) and white blood cells (WBC) and lymphocytes using an automatic blood analyser (ADVIA 120, Bayer, USA). Protein, albumin, and IgG concentrations were analysed in serum using an automatic biochemistry analyser (HITACHI 747, Japan). Ammonia nitrogen (NH₃-N) was measured in faeces according to the method of Chaney and Marbach (1992). Volatile fatty acids (VFA) and hydrogen sulphide (H₂S) were measured in faecal samples using a gaschromatograph and gas search probe, respectively. Statistical analysis was carried out applying a General Linear Model procedure (SAS[®] for Windows). Means were compared by Duncan's multiple range test.

Results and Discussion

Piglets of the PCF group had significantly higher feed intake and weight gain than piglets fed on the other diets. Feed conversion was slightly lower in the supplemented groups than in the control. Dry matter and protein digestibility measured on day 28 and 49 were slightly or significantly improved by the feed additives, while

there were no differences between treatments in digestibility measured on day 14. Noxious gas concentration in faeces of piglets was significantly lower in the FFC (H₂S) and the combination groups (NH₃-N, H₂S) on day 49. Reduction in NH₃-N concentration in faeces is in good agreement with better protein digestion in the PCF group. There were no differences, however, in faecal noxious gas volatile concentration on day 14 or 28 or in volatile fatty acid concentration (data not shown).

Table: Performance, digestibility, faecal gas concentration, and blood criteria

	NC	PC	NCF	PCF	SE
Average daily gain [g/d]	434 ^b	450 ^b	464 ^b	488 ^a	9
Average feed intake [g/d]	576 ^b	578 ^b	591 ^{ab}	613 ^a	9
Feed conversion [g/g]	1.33 ^a	1.28 ^a	1.27 ^a	1.26 ^a	0.03
Dry matter [%]					
Day 28	86.41 ^b	87.72 ^a	88.42 ^a	87.93 ^a	0.41
Day 49	87.74 ^a	89.09 ^a	88.83 ^a	89.91 ^a	0.75
Nitrogen [%]					
Day 28	87.66 ^b	87.40 ^b	88.37 ^a	88.85 ^a	0.35
Day 49	84.45 ^b	86.00 ^{ab}	87.01 ^{ab}	88.48 ^a	0.98
NH ₃ -N	871 ^a	738 ^{ab}	652 ^{ab}	619 ^b	70
H ₂ S	38.11 ^a	32.15 ^{ab}	28.14 ^b	28.98 ^b	2.5
RBC, x106/mm ³					
Day 1	5.28 ^a	5.10 ^A	4.45 ^a	5.08 ^a	0.279
Day 49	5.48 ^b	6.09 ^{ab}	5.83 ^{ab}	6.22 ^a	0.189
Difference	0.20 ^a	0.99 ^a	1.38 ^a	1.14 ^a	0.374
WBC, x103/mm ³					
Day 1	8.58 ^a	7.87 ^a	7.80 ^a	9.69 ^a	0.921
Day 49	9.39 ^b	12.68 ^{ab}	12.55 ^{ab}	17.15 ^a	1.53
Difference	0.81 ^a	4.81 ^a	4.75 ^a	7.46 ^a	2.3
IgG, mg/ml					
Day 1	268.6 ^a	249.6 ^a	256.4 ^a	270.8 ^a	7.34
Day 49	328.8 ^c	325.4 ^c	332.4 ^b	366.2 ^a	3.01
Difference	60.2 ^c	75.8 ^b	76.0 ^b	95.4 ^a	5.93

SE = pooled standard error, Values in a row with different superscripts differ at $P < 0.05$

Total protein and albumin concentration in serum of piglets were not influenced by dietary supplementation of FFC or AGP (data not shown). There was a significant increase in WBC during the experimental period (difference between day 1 and day 49) by the supplementation of FFC and AGP to the diets. Proportion of lymphocytes, however, was not influenced by AGP or FFC. The concentration of IgG was significantly increased by FFC. There was a synergistic effect of FFC and AGP on IgG in serum.

Summary

Feeding weaned piglets on diets supplemented with the phytogenic additive Fresta[®] F Conc. or in-feed antibiotics tendentially improved growth performance and increased digestibility of dietary protein. Improved performance was also shown in previous studies with plant based additives (Hong et al., 2004). Better protein digestibility was reflected in less $\text{NH}_3\text{-N}$ concentration in faeces. Faecal H_2S concentration was also slightly lowered by the feed additives. The dietary addition of Fresta[®] F Conc. or in-feed antibiotics led to numerical increase in white blood cells and serum IgG, while proportion of lymphocytes and blood protein were not increased. This might indicate an improvement in immune status of piglets. It is concluded that Fresta[®] F Conc. can be used to replace in-feed antibiotics and to reduce gaseous emissions in weaned piglets without negative effects on growth performance. Synergistic effects of antibiotics and Fresta[®] F Conc. on performance, digestibility and immune status show the potential of combining feed additives with different mode of action in order to improve health and performance in weaned piglets.

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Life Science - Herstellung prebiotischer Fleisch- und Wurstwaren

Production of prebiotic meat products and sausages

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Einführung

Aufgrund des bekanntermaßen oft zu geringen Verzehrs von Obst, Gemüse und Getreideprodukten kann für Ballaststoffe, Mineralstoffe und Vitamine eine Unterversorgung [1] verzeichnet werden, wodurch Erkrankungen wie Bluthochdruck, Diabetes, erhöhter Cholesterinspiegel oder Darmkrebs gefördert werden können. In den vergangenen Jahren ist daher ein steigendes Angebot an funktionellen Lebensmitteln mit gesundheitswirksamen Inhaltsstoffen zu verzeichnen. Für Fleisch- und Wurstwaren sind aber nur einige wenige Produkte erhältlich [2], was z.T. mit der Komplexität der technologischen Verfahrensführung zu begründen ist.

Da sich Fleisch- und Wurstwaren jedoch einer hohen Beliebtheit erfreuen, soll im vorliegenden Projekt über eine Zugabe von prebiotisch wirkenden Inhaltsstoffen der Gesundheitswert von Brüh-, Koch- und Rohwürsten erhöht und die technologische Verfahrensführung optimiert werden. In dieser Arbeit wird die Verwendung von Spirulina-Algen als Grobbestandteil in Brühwürsten untersucht. Dies umfasst die Arbeitsschritte: 1. Optimierung der technologischen Verfahrensführung und 2. Untersuchung der ernährungsphysiologischen Wirkung.

Die hier dargestellten Ergebnisse umfassen die Auswertung der technologischen Verfahrensparameter.

Material und Methoden

Eine herkömmliche Grundrezeptur für Brühwurst (75% Fleisch, 25% Fett, 20% Eis, 4% Schwartenemulsion, 0,5% Brühwurstgewürz, 2% NPS) wurde mit 0,3% Algen (*Earthrise®-Spirulina*, *Green Valley Naturprodukte Berlin*) angereichert. Das vorgekühlte gekühlte Fleisch und Fett wurde mit den anderen Rezepturbestandteilen im Kutter bis 12°C gekuttert. Mittels einer speziell entwickelten Verfahrensführung war es möglich, Algen als Grobbestandteile in das Brät einzugeben. Die Kontrollvariante entsprach der Grundrezeptur. Nach dem Abfüllen in Kaliber 60 Kunststoffdärme wurden die Würste einem üblichen Brüh- und Räucherregime unterzogen.

Untersucht wurden die Auswirkungen der veränderten Rezeptur auf die technologische Verarbeitungsfähigkeit der Bräte bzgl. Farbe (CIE-Lab) und Textur (PH - Penetrationshärte) sowie auf die Qualität der Fertigprodukte anhand von Farbe (CIE-Lab), Textur (PH - Penetrationshärte, TPA - Textur Profil Analyse mit Guillotine) und Sensorik (DLG-Prüfschema).

Ergebnisse und Diskussion

Nachfolgend sind die Brühwürste der Kontrollvariante und mit Algen als Grobeinlage dargestellt.

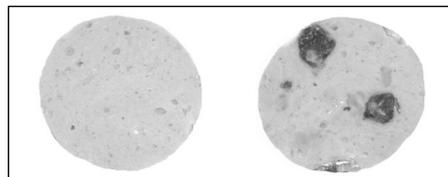


Abbildung 1. Brühwurst der Kontrollprobe (links) und Brühwurst angereichert mit Algen als Grobeinlage (rechts)

Die Resultate für die Texturparameter (Abb. 2) zeigen bzgl. der Penetrationshärte für Brät, dass für die Kontrollvariante eine höhere Festigkeit zu verzeichnen ist, was sich aber nicht in den Ergebnissen für die Brühwurst niederschlägt.

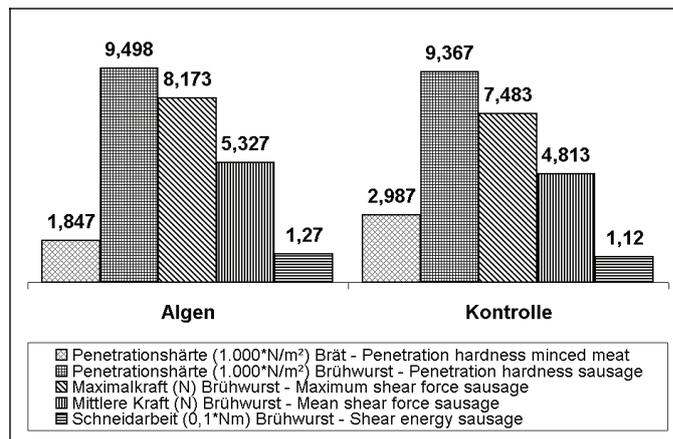


Abbildung 2. Texturparameter (PH, TPA) für Brät und Brühwurst
Figure 2. Texture parameters (PH, TPA) of minced meat and sausage

Für Brühwürste mit Algen als Grobeinlage wurde festgestellt, dass sowohl für die Penetrationshärte als auch die TPA eine tendenziell festere Textur registriert wurde. Dies wurde durch die Konsistenz der Algen-Grobbestandteile bedingt.

Durch die grünen groben Algenbestandteile war ein direkter Vergleich der Farbwerte für das Brät als auch die Brühwurst (Tab. 1) mit der Kontrollvariante nicht möglich. So wurde für die Messungen nur das Grundbrät betrachtet, um Aussagen darüber zu erhalten, ob Farbbestandteile aus den Algeneinlagen in das Brät übergehen. Nach ersten Erkenntnissen scheint dies nicht der Fall zu sein, was als positiv für die Produktqualität bzgl. Aussehen zu bewerten ist.

Tabelle 1. Farbwerte (CIE-Lab) für Brät und Brühwurst
Table 1. Colour traits (CIE-Lab) of minced meat and sausage

	Brät (Minced meat)		Brühwurst (Sausage)	
	Algen	Kontrolle	Algen	Kontrolle
L (Lightness)	61,43	67,63	63,04	65,28
a (Redness)	3,41	4,56	9,66	9,13
b (Yellowness)	16,96	17,8	13,42	13,30
c (Saturation)	17,3	18,38	16,53	16,14
h (Hue angle)	78,63	75,62	54,25	55,52

Die sensorische Prüfung nach DLG-Prüfschema (Abb. 3) zeigt, dass beide Varianten nicht einer optimalen Brühwurst gleich kommen. Die Konsistenz wurde bei beiden Varianten gleichwertig beurteilt. Geschmacklich wurde die Brühwurst mit Algeneinlage besser bewertet als die Kontrollvariante, da eine geringere Schmalzigkeit und Fettigkeit festgestellt wurde. Negativ zu bewerten war die Brühwurst mit Algeneinlage bzgl. äußerer Kriterien und Aussehen aufgrund von Hohlräumen unter der Hülle, da sich diese ablöste.

Eine Verbraucherumfrage mit Verkostung zeigte, dass 79% die Brühwurst mit Algeneinlage und 71% die Brühwurst der Kontrollvariante konsumieren würden (n=228, Mehrfachnennung).

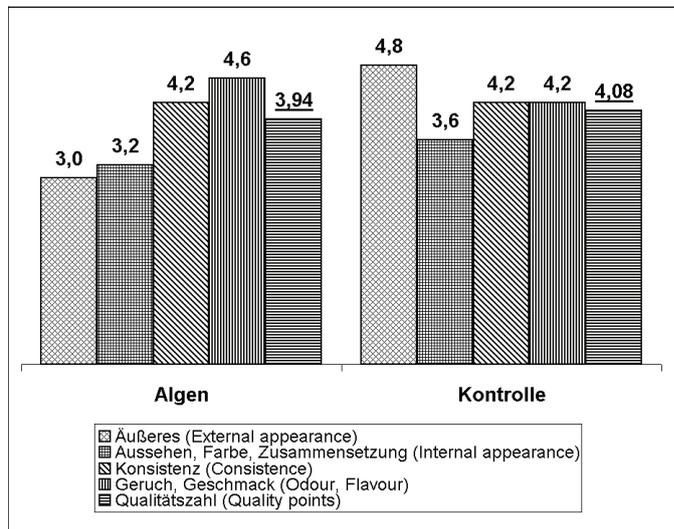


Abbildung 3. Sensorik nach DLG-Prüfschema für kalte Brühwurst (n=5)
Figure 3. Sensory analysis by DLG test procedure of cold sausage (n=5)

Summary

The claim of the project is the production of new prebiotic sausages. By development of a new technology it's possible to add algae as coarse pieces in minced meat. As a result, the quality of conventional sausages (control) was partially improved. The texture (Penetration hardness and Textur profile analysis) appeared to be less soft than control. Sensory analysis by DLG test procedure showed that sausage with algae is less greasy and fatty, also better in the flavour and odour than control. Next steps are to optimize the technology to add algae as coarse pieces and improve the recipe of minced meat, also examine the dietetical/physiological effects of sausages with added algae.

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Influence of probiotics on immunological parameters of healthy volunteers and patients with atopic dermatitis

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Introduction

The supporting role of probiotics in the prevention and therapy of atopic diseases is widely described. The effect is based on the interaction of the colonic microflora with the gut associated lymphoid tissue. Aberrant gut microbial compositions have been found in patients with atopic dermatitis (Björkstén et al. 1999; Kirjavainen et al. 2001). Phagocytic receptor activity of granulocytes, as a marker for unspecific cellular immune response, is downregulated in these patients whereas a stimulation is observed in healthy subjects (Pelto et al. 1998).

In the present study we investigated the effects of *Lb. paracasei* LPC37, *Lb. acidophilus* 74-2 and *Bf. species* 420 on the immune system of 15 healthy subjects and 15 patients with moderate atopic dermatitis.

Materials and Methods

The study was designed as a placebo-controlled and cross-over trial. During the initial period (3 weeks) participants had to eliminate probiotic products from their daily diet. All participants were clinically examined prior to the study. Healthy volunteers had to be free of allergies, allergic rhinitis and asthma. Inclusion criteria for patients were moderate atopic dermatitis with Scoring Atopic Dermatitis (SCORAD) to range between 5 and 30. Sensitization for cow's milk was excluded by skin prick test with commercial allergen extract.

At the beginning volunteers were randomized into two groups. They consumed twice a day either 100 ml/d probiotic or placebo yoghurt drink over 8 weeks. After a 2 week wash-out period intervention changed between the groups. Blood samples were collected at the beginning and at the end of each period.

For analysis of selected clusters of differentiation (CD57, CD54) heparinized blood was incubated with phycoerythrin-conjugated detection antibodies according to the manufacture's instructions (BD Biosciences, USA). Cells were measured via flow cytometry (FACScan, CellQuest software)

Phagocytic activity of granulocytes was determined using the commercial test kit Phagotest®(ORPEGEN, Germany). The percentage of granulocytes that ingested FITC-labelled opsonized *Escherichia coli* was detected.

Results

Parameters of specific and unspecific immune response significantly changed during the study. The expression of natural killer cells (CD57) increased significantly compared to placebo in healthy subjects. No influence could be shown for patients with atopic dermatitis (Table 1).

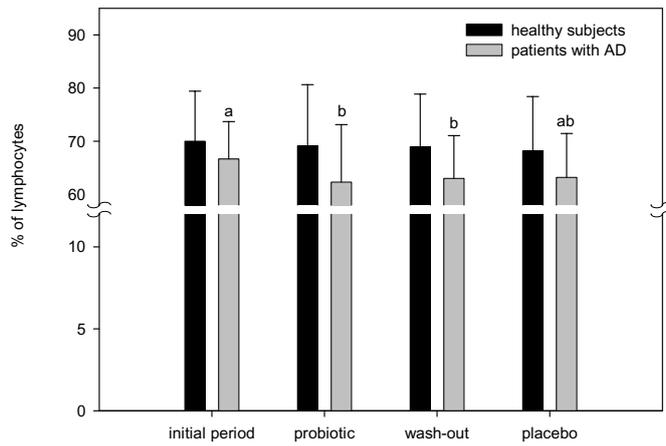
Table 1. Expression of natural killer cells (CD57)

	Initial period	Probiotic	Wash-out	Placebo
Healthy subjects	7.1 ± 5.8	8.7 ± 5.2 ^a	5.7 ± 3.1 ^b	5.3 ± 3.7 ^b
Patients with atopic dermatitis	6.5 ± 7.6	6.3 ± 4.4	6.9 ± 5.0	6.7 ± 3.5

a, b = significant differences between mean values of unequal superscripts (p < 0.05)

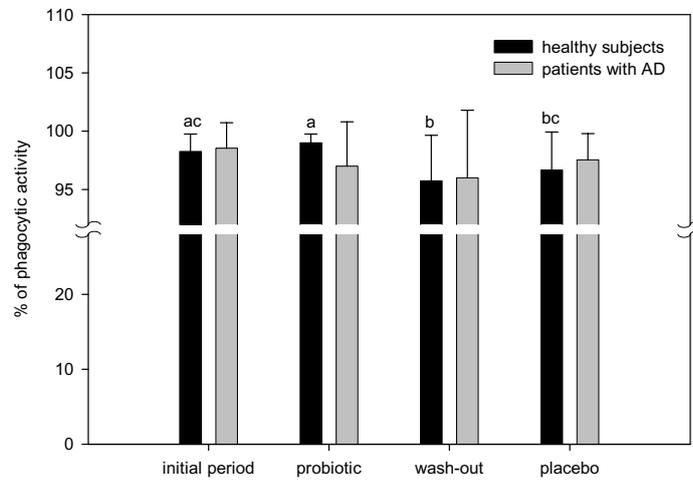
For the expression of intercellular adhesion molecule-1 (ICAM-1, CD54) a significant decrease after probiotic intervention versus initial period was detected in atopic patients, whereas in healthy subjects no alteration was observed (Figure 1).

The phagocytic activity of granulocytes and monocytes as a marker for immune system stimulation was increased during probiotic intervention and decreased significantly after cessation (Figure 2).



a, b = significant differences between mean values of unequal superscripts ($p < 0.05$)

Figure 1. Expression of ICAM-1 (CD54)



a, b c = significant differences between mean values of unequal superscripts ($p < 0.05$)

Figure 2. Phagocytic activity of granulocytes and monocytes

Discussion

Results demonstrate that probiotic cultures influence parameters of specific and unspecific immune response in different ways. Increased expression of natural killer cells and increased phagocytic activity of granulocytes and monocytes after intervention with probiotic drink containing *Lb. paracasei* LPC37, *Lb. acidophilus* 74-2 and *Bf. species* 420 point to a stimulation of immune system in healthy subjects. Gill et al. (2001) showed enhanced activity of natural killer cells in the elderly after intake of *Lb. rhamnosus* GG. Similar effects were observed after micronutrient supplementation (Santos et al. 1998). This indicates that immunity is affected by dietary intake of additives (e.g. probiotics, vitamins). The increase in phagocytic activity refers a stimulation of the immune system owing to the probiotic cultures. This effect is in conformity with results of other studies (Jahreis et al. 2002, Gill et al. 2001, Arunachalam et al. 2000).

The expression of intercellular adhesion molecule-1 (ICAM-1, CD54) is increased during inflammation, virus infection and cellular stress. In the present study ICAM-1 was significantly downregulated after probiotic consumption in patients with atopic dermatitis. Thus, these findings reveal a beneficial effect of probiotic cultures in both, patients with atopic dermatitis and healthy subjects.

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On the influence of β -glucans from *Saccharomyces cerevisiae* on sow and litter performance during lactation

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Introduction

In search of alternatives for antimicrobial growth promoters various feed additives are used in farm animal feeding to improve animal health and performance. Among these are 1,3/1,6- β -D-glucans extracted from yeast cell walls. They are supposed to stimulate the immune system thus enhancing animal performance as it was shown in previous studies with various types of animals (Engstad and Raa 1999).

Material and Methods

To prove this, a trial was conducted feeding yeast cell wall extracts to 15 sows from a commercial herd between d112 of gestation and weaning (d21). The animals were divided into 3 groups of 5 sows each in the experiment and housed into individual farrowing crates. Diets consisted of a standard barley-soybean meal (13.0 MJ ME/kg, 17.5 % XP) and were supplemented with 0 g (control), 4 g (group 1) or 2.2 g (group 2) β -glucan per sow and day, respectively. From d112 of gestation until farrowing sows were fed restrictively with 1.5 kg of the lactation diet which was then increased daily by 700 g until *ad libitum* intake from d6 of lactation on. Creep feed was offered to suckling piglets starting on d10 after birth. Sows and piglets had free access to tap water.

Results and discussion

Significant treatment effects on litter performance were not observed (table 1). The number of piglets born alive varied insignificantly between 10.2 (control group) and 12.2 (group 2). However, these differences were not due to β -glucan-supplementation so that cross-fostering occurred within 24 h *post partum*, irrespec-

tive of treatment. Cross-fostering is an usual method to compensate for unequal litter weight resp. size at birth and was carried out by Fleischer et. al. (2001) and Auerbach and Ranft (2001) during their experiments as well (table 2). Nonetheless, there was a positive tendency towards higher litter weight at weaning for group 1 amounting to 11.9 kg compared to the control group ($p = 5,6 \%$). Moreover, litter weight gain was 8.5 kg higher than in the control group ($p > 0.05$).

Table 1: Effects of β -glucans added to a lactation diet on litter performance

β -glucan content per kg feed Litters (n)	Control - 5	Group 1 4 g 5	Group 2 2.2 g 5
Number of	0 s	0 s	0 s
Piglets born alive	10.2 \pm 1.3	11.2 \pm 2.3	12.2 \pm 3.4
Piglets born dead	1.6 \pm 0.9	0.6 \pm 1.3	0.8 \pm 1.1
Weaned piglets	8.2 \pm 0.8	9.4 \pm 1.1	9.8 \pm 1.6
in kg:			
Piglet weight gain	4.3 \pm 0.5	4.7 \pm 0.7	4.0 \pm 0.3
Litter weight at birth	14.4 \pm 2.2	17.8 \pm 1.8	16.7 \pm 5.5
Litter weight at weaning	47.3 \pm 10.1	59.2 \pm 6.3	53.0 \pm 9.9
Litter weight gain	32.9 \pm 10.0	41.4 \pm 7.3	36.2 \pm 9.8

$p > 0.05$

As it is shown in the review presented in table 2, Auerbach and Ranft (2001) recorded significantly higher litter weight at weaning in the β -glucan groups compared to an unsupplemented control group. This result was confirmed by the experiments of Fleischer et al. (2001). In our own experiments we received the same results at least in tendency.

As to the mechanisms lying beyond increased performance in β -glucan supplemented animals ADEREM and ULEVITCH (2000) showed that β -glucans attach to specific receptors on macrophages thus stimulating antibody-producing B-lymphocytes and other cells of the immune system. This thesis is supported by DECUYPERE et al. (1998). The authors observed a higher amount of antibodies in sows' milk which were fed β -glucans compared to an unsupplemented control group when both groups were vaccinated against *E. coli* 3 weeks *ante partum* (a.p.).

Moreover it may be possible that feeding β -glucans to sows had an immune-stimulating effect which lead to an improved well-being of the animals. Followingly,

feed intake may have increased thus leading to a higher milk yield in sows. This may be an explanation for increased piglet weight at weaning in β -glucan supplemented groups. However, as our trial was carried out under practical farming conditions it was impossible to determine sows' feed intake or milk yield.

Table 2: Influence of β -glucans on sow performance (review)

	Decuyper et al. (1998)	Auerbach and Ranft (2001)	Auerbach and Ranft (2001)	Fleischer et al. (2001)
Product	MacroGard®	Not specified	Not specified	Leucogard®
yeast	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>
Dosage per sow and day (g)	2.5	8	8.75	7.5
administration	orally	orally	orally	orally
No. of animals (n)	34	274	79	20
Trial start (day of gestation)	108 th	107 th -109 th	108 th -110 th	108 th
Trial duration (d)	21	28	24	24
Further treatments	<i>E. coli</i> vacc 3 weeks a.p.	Cross- fostering	Cross- fostering	Cross- fostering
Performance: trial group vs. control group				
Number of				
Piglets born alive	K 10.1 V ± 0	10.3 +0.1	11.4 ± 0	11.1 -0.3
Piglets born dead	K 0.2 V +0.2	0.6 +0.05	1.0 +0.05	n.d. n.d
Weaned piglets	K n.d. V n.d	9.0 +0.6 p<0.02	8.2 +0.7 p<0.05	8.1 +1.1
Litter weight at weaning (kg)	K n.d. V n.d	54.5 +5.2 p<0.02	37.8 +8.2 p<0.01	25.8 +5.6 p<0.01
Piglet weight at weaning (kg)	K 4.5 V -0.6 p<0,01	6.0 +0.2	4.7 +0.5 p<0.05	4.8 +0.4

K: control group; V: trial group; n.d.: not determined

Summary

In a commercial herd fifteen sows were divided into three groups of five animals each from day 112 of gestation on. All animals received the same lactation feeding which was supplemented with 0 g (control), 4 g (group 1) or 2.2 g (group 2) β -glucan per sow and day until weaning (d21). There was a positive influence on litter weight at weaning and litter weight gain in the β -glucan supplemented groups. Further research is necessary concerning the use of 1,3/1,6- β -D-glucans to understand the

immunological mechanisms forming the basis of this amelioration e.g. in view of antibody contents in sows' milk.

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Einfluss von Flavomycin® und BioPlus 2B auf das Aufzuchtergebnis bei Kälbern

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Zielstellung

In den vergangenen Jahren erfolgte zur Verbesserung der Mikroflora im Verdauungstrakt bei den Kälbern weitgehend ein Austausch der Fütterungsantibiotika durch Probiotika.

In einem Einzelfütterungsversuch mit 40 Kälbern sollte der Einfluss eines Probiotikums (BioPlus 2B) vergleichend gegenüber mit Flavomycin behandelten Kälbern auf Futteraufnahme, Lebendmasseentwicklung, Tiergesundheit und ausgewählte Blutparameter geprüft werden.

Lösungsweg

Es wurden 40 männliche Kälber der Rasse „Schwarzbuntes Rind“ eingestallt. Die Tiere hatten zu Versuchsbeginn ein mittleres Alter von 21 - 28 Tagen. In 28-tägigen Abständen wurden Kontrollwägungen durchgeführt. Neben dem Milchaustauscher (maximale Menge 110 g/l und 7 l Tränkmenge) gelangte Kälberaufzuchtfutter ad libitum, Trockengrünhäcksel und Stroh bis maximal 0,7 kg pro Tier und Tag zum Einsatz. Entsprechend des Alters und des Trockenfuttermittels reduzierte sich die Milchaustauschermenge.

Gruppeneinteilung:

Gr. 1: Flavomycin; 10 mg/kg Milchaustauscher

Gr. 2: BioPlus 2B; 400 mg/kg Milchaustauscher

Vor jeder Mahlzeit erfolgte die Registrierung der Durchfälle und anderer Krankheitssymptome. Je nach Durchfallstärke wurde die Tränkmenge reduziert. In schweren Fällen erfolgte eine Behandlung durch den Tierarzt.

Entsprechend des vorgegebenen Versuchsplanes wurde ausgewählten Tieren (5 je Gruppe) zu Versuchsbeginn und zu Versuchsende Blut aus der Vena jugularis entnommen. Mit Hilfe eines Blutzellzählgerätes erfolgte die Bestimmung verschiedener Blutparameter: Erythrozyten, Hämatokrit, mittleres Erythrozytenvolumen, mittlerer

Hämoglobingehalt pro Erythrozyt, mittlere Hämoglobinkonzentration in den Erythrozyten, Leukozyten, Thrombozyten und Hämoglobin.

Tabelle 1: Mittlere tägliche Aufnahme an Trockenmasse, Rohprotein, Energie und mittlere Lebendmassezunahme der Kälber (1. - 28. bzw. 1. - 56. Versuchstag; alle Parameter: $p > 0,05$)

Gruppe Präparat Dosierung (mg/kg MAT)	1 Flavomycin 10	2 BioPlus 2B 400
1. - 28. Versuchstag		
Durchfalltage/Tier	2,64	2,90
Trockenmasse (kg)	1,25 ∇ 0,24	1,10 ∇ 0,23
Rohprotein (g)	266 ∇ 50	235 ∇ 47
Umsetzbare Energie (MJ)	18,10 ∇ 2,90	16,30 ∇ 2,70
Lebendmassezunahme (g/Tier/Tag)	606 ∇ 170	548 ∇ 215
1. - 56. Versuchstag		
Durchfalltage/Tier	3,41	3,48
Trockenmasse (kg)	1,61 ∇ 0,26	1,46 ∇ 0,30
Rohprotein (g)	338 ∇ 54	307 ∇ 62
Umsetzbare Energie (MJ)	21,80 ∇ 3,10	20,10 ∇ 3,60
Lebendmassezunahme (g/Tier/Tag)	776 ∇ 155	713 ∇ 202

Ergebnisse

Der Zusatz von BioPlus 2B hatte keinen wesentlichen Einfluss auf die Futtermittelaufnahme in den einzelnen Versuchsabschnitten bzw. der gesamten Versuchsperiode (Tab. 1 und 2).

Im Mittel des gesamten Versuchszeitraumes lag die tägliche Aufnahme an umsetzbarer Energie bei 25,65 MJ/Tier und Tag. Der mittlere Rohproteinverzehr kann mit 415 g/Tier/Tag angegeben werden.

Die Tiere beider Varianten wiesen im gesamten Versuchszeitraum ein hohes Zunahmestadium aus (Tab. 1, 2 und Abb. 1). Sowohl für den Zeitraum 1. - 28. Versuchstag als auch über die gesamte Versuchsperiode (1. - 84. Tag) nahmen die Kälber der Versuchsgruppe (BioPlus) im Trend gegenüber den Tieren der Kontrollgruppe (Flavomycin) ca. 10 % bzw. 7 % weniger zu (Abb. 1; $p > 0,05$). Aufgrund annähernd gleicher Nährstoffaufnahme und Lebendmassezunahme der Kälber in beiden Gruppen ergeben sich für die Aufwandswerte nur geringe Differenzen (Tab. 1 und 2). Der Energieaufwand kann im Mittel beider Gruppen mit 28,57 MJ ME/kg LMZ angegeben werden und steht in Übereinstimmung mit anderen Untersuchungen im ent-

sprechenden Lebendmasseabschnitt der Kälber. Die Durchfallhäufigkeit konnte in diesen Untersuchungen durch BioPlus 2B (Gr. 2) um ~9,5 % gegenüber der Flavomycinbehandlung gesenkt werden ($p > 0.05$; Tab. 2).

Tabelle 2: Aufzuchtparameter über den gesamten Versuchszeitraum (1. - 84. Versuchstag; alle Parameter $p > 0,05$)

Gruppe	1	2
Lebendmassezunahme (g)	929 ∇ 130	866 ∇ 154
Aufnahme		
Trockenmasse (kg)	2,07 ∇ 0,22	1,92 ∇ 0,29
Rohprotein (g)	431 ∇ 45	399 ∇ 58
Umsetzbare Energie (MJ)	26,60 ∇ 2,60	24,70 ∇ 3,30
Aufwand (je kg LMZ)		
Trockenmasse (kg)	2,23 ∇ 0,15	2,22 ∇ 0,19
Umsetzbare Energie (MJ)	28,63 ∇ 2,1	28,52 ∇ 2,4
Durchfallhäufigkeit (Tage/Tier)	4,2	3,8

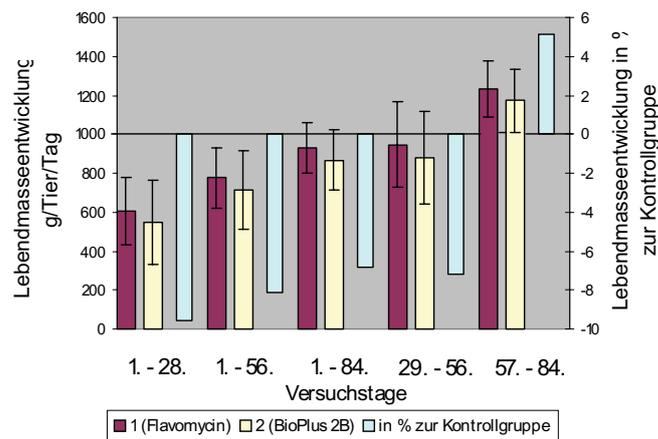


Abbildung 1: Lebendmasseentwicklung in den einzelnen Versuchsabschnitten (g/Tier/Tag; in % zur Kontrollgruppe)

Die geprüften Stoffwechsel- und Blutbildparameter befinden sich in dem für Kälber dieser Altersgruppe angegebenen Normbereich (Tab. 3).

Tabelle 3: Einfluss beider Präparate auf ausgewählte Blutbild- und Stoffwechselparameter ($p > 0,05$)

Gruppe Präparat	1 Flavomycin	2 BioPlus 2B
Erythrozyten ($\times 10^6/\text{mm}^3$)	9,29	9,99
Hämatokrit (%)	38,5	38,1
Hämoglobin (g/dl)	9,5	9,3
mittlere Hb-Konzentration in den Erythrozyten (%)	27,1	26,4
Leukozyten ($\times 10^3/\text{mm}^3$)	11,0	9,9
Thrombozyten ($\times 10^3/\text{mm}^3$)	240	215
Aspartatamintransferase (n kat/l Serum)	816	852

Fazit

In einem Einzelfütterungsversuch mit je 20 Kälbern/Gruppe wurde der Einfluss von BioPlus 2B (Probiotikum) vergleichend gegenüber Kälbern mit Flavomycinzusatz auf die Aufzuchtergebnisse, Tiergesundheit und Blutparameter geprüft. Die Tiere beider geprüfter Varianten weisen über den gesamten Versuchszeitraum ein hohes Leistungsniveau auf (898 g Lebendmassezunahme je Tier und Tag). Unter den von uns geprüften Bedingungen übte BioPlus 2B eine um 7 % geringere Lebendmassezunahme gegenüber den Kälbern mit der Flavomycinzulage aus ($p > 0,05$). Die Durchfallhäufigkeit der Kälber mit BioPlus war im Trend um ca. 10 % geringer. Alle geprüften Blutparameter befinden sich im Normbereich.

Summary

The effects of a probiotic additive (BioPlus 2B; 400 mg/kg milk replacer; group 2) in contrast to Flavomycin treated calves (10 mg/kg milk replacer, group 1) on feed intake, live weight, animal health and different parameters of blood were tested in a feeding trial involving 40 calves. At the beginning of the trial the calves were 21 - 28 days old. In addition to the milk replacer (maximum 110 g/l water and a quantity of 7 l) they were fed with growing feed for calves ad libitum as well as dried chopped grass and straw (maximum 0.7 kg/animal per day).

The group treated with BioPlus 2B shows a lower live weight gain of about 7 % in contrast to the Flavomycin treated group over the whole experimental period (1. - 84. day of trial), in the first 28 days even a 10 % lower live weight gain ($p > 0.05$). All measured blood parameters showed normally values and were not affected by the preparation.

Auswirkungen einer BioPlus 2B-Supplementation bei Ferkeln

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Einführung

Die Ferkelhalter benötigen zur Gesundheits- und Leistungsstabilisierung der Ferkel Futterzusätze, die eine deutliche reproduzierbare Wirkung haben, Verbraucherakzeptanz genießen und ökonomisch vorteilhaft sind. Unter verschiedenen Bedingungen sind neue Futtersupplemente für Beratung und Praxis zu prüfen. Nicht in allen Experimenten nahmen die getesteten Probiotika einen positiven Einfluss auf die zootechnischen Leistungen und den Gesundheitsstatus der Tiere (Beck und Hackl, 2002; Bolduan und Hackl, 1995; Gedek, 1991; Heugten et al., 2003; Kühn, 1998; Linder Mayer und Probstmeier, 1995; Overland et al., 2003; Simon, 2001; Wetscherek, 2002).

Ziel der Fütterungsversuche war die Testung des probiotischen Produktes „BioPlus 2B“ bei Ferkeln.

Material und Methoden

In drei Versuchen mit 126 Börgen im Alter von 28 - 70 Tagen in Flatdeck-Haltung mit Spaltenboden wurde das Probiotikum „BioPlus 2 B“ eingesetzt. BioPlus 2 B ist eine Mischung aus *Bac. licheniformis* und *Bac. subtilis* im Verhältnis 1:1 und wurde 0,4 g/kg Mischfutter im gesamten Versuchszeitraum supplementiert. Die Ferkel der Kontrollgruppe erhielten keinen „Leistungsförderer“ ins Futter. Die Ferkel bekamen in der zweiphasigen Aufzucht in der 5. und 6. Lebenswoche ein pelletiertes Ferkelstarter- und von der 7.-10. Lebenswoche ein pelletiertes Ferkelaufzuchtfutter ad libitum (Tab. 1 und 2). Das Mischfutter im Versuch C war ähnlich wie im Versuch B zusammengesetzt. Die Analysen der Mischfutter ergaben keine wesentlichen Unterschiede zwischen den Gruppen je Versuch.

Im Versuch A kamen die Genotypen Pi x DE bzw. DL x DE und in den Versuchen B und C PiHa x C23 (PIC) zum Einsatz. Je Gruppe der Versuche A und B wurden sechs Wiederholungen und im Versuch C 9 Wiederholungen mit je drei Ferkeln mit einer Körpermasse von 8,0 kg (Versuch A), 8,2 kg (Versuch B) bzw. 7,1 kg (Versuch C) eingestallt. Am Versuchsbeginn, am 14. und am 42. Versuchstag erfolgte die Einzelwägung der Ferkel. Am Ende der zweiten Versuchswoche und am Versuchsende wurde zur Ermittlung des Futtermittelsverzehrs die Rückwaage der Futterreste vorgenommen. Für die statistische Bewertung der Versuchsergebnisse sind die Standardabweichung und der Tukey-Test herangezogen worden.

Ergebnisse und Diskussion

Die Ergänzung des Ferkelfutters mit BioPlus 2 B nahm keinen gerichteten Einfluss auf die zootechnischen Leistungen (Tab. 3). In der fünften und sechsten Lebenswoche verzehrten die Ferkel im Mittel der drei Versuche bei Zusatz von BioPlus 2 B 5,4 % mehr Futter als die Kontrolltiere ohne Zusatz. Von der siebenten bis zur zehnten Lebenswoche lag der Futter-

verzehr in den beiden Gruppen gleich. Daraus ergibt sich ein nicht signifikanter Mehrverzehr in der sechswöchigen Versuchsperiode von 0,7 % bei Supplementation von BioPlus 2 B.

Tabelle 1: Zusammensetzung und wertbestimmende Inhaltsstoffe in g/kg Mischfutter (Versuch A)

Komponenten/Gehalte	Ferkelstarter-		Ferkelaufzuchtfutter	
	ohne	mit BioPlus	ohne	mit BioPlus
Weizen		195		235
Zwiebackbruch		200		120
Gerste		100		260
Weizengrießkleie		-		30
Sojabohnen		75		23
Sojaextraktionsschrot, 49		175		150
Süß-Molkepulver		60		50
Magermilchpulver		60		30
Molke-Fett-Konzentrat		20		20
Mirakel 1-35 FE		35		10
Sojaoel		30		27
Mineralfutter		50		45
	ohne	mit BioPlus	ohne	mit BioPlus
Energie (MJ ME)	15,4	15,4	14,9	14,7
Rohprotein	216	213	189	190
Rohfaser	21	23	27	27
Rohfett	97	97	75	75
Rohasche	60	60	56	54
Stärke	280	276	359	339
Calcium	8,6	8,6	8,5	8,5
Phosphor	6,7	6,7	6,4	6,4

Im Mittel der drei Versuche war die Körpermasseentwicklung analog der aufgenommenen Futtermenge. Die Ferkel nahmen in der Starterperiode bei BioPlus-Ergänzung 7,9 % mehr an Körpermasse zu als die Kontrolltiere, allerdings statistisch nicht zu sichern. Da die Ferkel mit BioPlus 2B in der 7. - 10. Lebenswoche weniger zunahmen als die Kontrolltiere, bestehen in der sechswöchigen Ferkelaufzucht keine Unterschiede zwischen den Kontroll- und Versuchsgruppen (438 g vs. 437 g).

Im Futteraufwand je kg Zunahme lagen die „BioPlus“-Ferkel in den ersten zwei Versuchswochen im Mittel der Versuche wiederum 2,5 % günstiger als die Kontrolltiere. Aber in der Gesamtperiode bestanden keine Unterschiede zwischen der Kontrollgruppen ohne Zusatz und den BioPlus-Gruppen (1,37 vs. 1,38).

Die Ergebnisse zeigen, dass die Probiotika-Ergänzung der Ferkelstarterfutters 5,4 % Mehrverzehr, 7,9 % bessere Zunahmen und 2,5 % günstigere Futtermittelverwertung brachte. Leider konnten die guten Ergebnisse nicht in dem Abschnitt 7. - 10. Lebenswoche erreicht werden. Die positiven Resultate in der Gesamtperiode des Versuches A konnten in den folgenden Versuchen B und C nicht bestätigt werden. Die Versuche verliefen ohne Störungen und der Gesundheitsstatus der Ferkel war gut.

Tabelle 2: Zusammensetzung und wertbestimmende Inhaltsstoffe in g/kg Mischfutter (Versuch B)

Komponenten/Gehalte	Ferkelstarter-		Ferkelaufzuchtfutter	
	ohne	mit BioPlus	ohne	mit BioPlus
Weizen		210		250
Waffelmehl		200		125
Gerste		100		260
Weizengrießkleie		-		30
Sojabohnen		75		23
Sojaextraktionsschrot, 49		180		150
Süß-Molkepulver		60		50
Magermilchpulver		60		30
Molke-Fett-Konzentrat		20		20
Mirakel 1-35 FE		35		10
Bergafat HTL 306		8		6
Sojaoel		7		6
Mineralfutter		45		40
	ohne	mit BioPlus	ohne	mit BioPlus
Energie (MJ ME)	14,8	14,7	13,9	14,2
Rohprotein	218	218	189	188
Rohfaser	23	24	30	30
Rohfett	85	80	58	62
Rohasche	63	62	54	56
Stärke	270	266	333	345
Calcium	10,9	10,6	9,0	9,5
Phosphor	7,3	7,1	5,9	6,1

Die Tierabgangsrate und das Durchfallgeschehen der Ferkel in den Versuchen blieb von der Fütterung unbeeinflusst, wobei in den supplementierten Gruppen die Tendenz zu niedrigerer Durchfallrate und -intensität bestand (Tab. 3). Auch Lindermayer (2003) konnte bei Zusatz von 0,4 g BioPlus 2B/kg Futter in zwei Versuchen keine deutlichen Änderungen der Aufzuchtleistungen und des Gesundheitszustandes der Ferkel nachweisen.

Summary

Effects of BioPlus 2B supplementation on piglets

In three experiments the effect of the probioticum "BioPlus 2B" was investigated on 126 fattening castrates during the 28 – 70 day of life. On the average of the three experiments, the supplementation of starter diet with Bio Plus 2B improved feed intake by 5.4 %, life weight gain by 7.9 %, and feed efficiency by 2.5 % during the 5th and 6th week of life. However, the differences remained insignificant. When the whole period of the experiment was regarded the life weight gain was reduced by 0.2 % and the feed efficiency was impaired by 0.7 %. From the economical point of view no improvement was observed during the 6 weeks of investigation.

Literatur

(liegt beim Verfasser vor)

Tabelle 3: Leistungen von Ferkeln ohne und mit BioPlus 2B-Zusatz

Parameter	Versuch A			Versuch B			Versuch C		
	ohne Zusatz		mit BioPlus 2B	ohne Zusatz		mit BioPlus 2B	ohne Zusatz		mit BioPlus 2B
	\bar{x}	s ±	\bar{x}	\bar{x}	s ±	\bar{x}	s ±	\bar{x}	s ±
Futterverzehr, g/Tier + Tag									
1. - 14. d	219	26	242	290	35	277	222	48	224
15. - 42. d	836	95	850	798	67	813	725	86	710
1. - 42. d relativ	631	65	648	628	49	635	556	68	548
	100		102,7	100		101,1	100		98,6
Körpermassenzunahme, g/Tier + Tag									
1. - 14. d	197	62	251	242	91	232	175	83	195
15. - 42. d	596	67	622	578	95	567	515	106	486
1. - 42. d relativ	463	57	497	466	82	456	401	90	389
	100		107,3	100		97,9	100		97,0
Futteraufwand, kg/kg Zunahme									
1. - 14. d	1,10	0,26	0,97	1,20	0,08	1,20	1,27	0,34	1,15
15. - 42. d	1,41	0,14	1,37	1,38	0,04	1,43*	1,41	0,08	1,46
1. - 42. d relativ	1,36	0,12	1,30	1,35	0,02	1,39*	1,39	0,06	1,41
	100		95,6	100		103,0	100		101,4
Tierabgänge, %	0		0	0		0	7,4		0
Durchfalliere, %	5,6		0	5,6		5,6	0		0
Durchfallbehandlung/ behandeltes Tier	3		0	1		1	0		0

* signifikant zur Gruppe ohne Zusatz, p < 0,05

The *in vitro* effect of chromium Cr⁺³ and selenium (Se⁺⁴ or Se⁻²) ions on carnitine uptake and fatty acids β -oxidation efficiency in rat's lymphocytes.

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Introduction

Carnitine, (betaine of β -hydroxyl- γ -trimethylamino-butyrac acid), is an essential component of the system of fatty acid trans-membrane transport and its degradation in mitochondria. This compound, often in combination with chromium picolinate, is an ingredient of weight loss food supplement. Chromium is needed for proper insulin function causing improvement of tissue insulin sensitivity. Davis et al. (1996, 1997) demonstrated that Cr⁺³ stimulates activity of tyrosine kinase, which is a part of insulin receptor.

Chromium is trace element affecting, among others, glucose uptake by rat's erythrocytes (Lipko et al., 2002, 2003) and β -oxidation activity in broiler chicken and healthy rat's lymphocytes (Kuryl et al., 2002, 2004). Supplementation of the diet of swine with chromium, used in the form of chromium yeast resulted in lowering of the level of fat in the carcass (Sawosz, 1999). This factor is also effective in stimulation of these metabolic pathways of sugars and fatty acids in animals fed with mild energy restricted diet (Kuryl et al., 2003, Krejpcio et al., 2004). The benefits from such modified diet, often supplemented with carnitine, are systemic effects by modulation of lipids metabolism and improved absorption of Ca, Mg and Fe ions, accompanied in humans by lowering of fat content as well as body weight reduction. (Brighentin et al., 1999, Letexier et al., 2003)

Selenium deficiency in humans manifests as Keshan disease, Kashin-Back disease and several kind of miopathies (Ermakov, 2004), what may suggest the potential participation of this ion in energetical process regulation. The reason of these pathologies is very low selenium level in the soil resulting in very low content of this essential trace element in food. The biologically active forms of selenium in organisms are glutathione peroxidase, 5'iodotyrosine deiodase, thioredoxine reductase and seleno-protein P. Toxicity of selenium is discussed. Selenium intake recommendation were well known but the form of this supplementation are not taken into account. However, metabolic effects of various selenium oxidation state and compounds are often quite different. In plant fertilization are used inorganic forms - sodium selenite and/or sodium selenate mainly as additives of microelement fertilizers applied on leaves or

directly to the soil Debski et al., 2001). In organisms selenium is mostly present in the form of seleno-methionine and/or seleno-cysteine.

These studies were undertaken with the aim study the role of chromium Cr^{+3} , selenium Se^{+4} and Se^{-2} in carnitine uptake and following effect on β -oxidation of fatty acids by lymphocytes of healthy rats.

Materials and Methods

Experiments were performed on lymphocytes isolated from blood of healthy Wisteria rats. Rats were 4-weeks old and weight 130 - 150 g. Lymphocytes were isolated by centrifugation on FICOLL - Hypaque 1077. The layer of lymphocytes was collected, resuspended in phosphate buffered saline (PBS) and recentrifuged for washing from FICOLL. Final pellet of lymphocytes was resuspended in Hank's Balanced Salt Solution (HBSS) to a final concentration of protein of about 1 mg/ml.

Carnitine uptake was measured at 37°C for 60 min. in shaking water bath in reaction mixtures contained lymphocytes (20 - 50 μg of protein) and 50 μM carnitine in a final volume of 250 μl . After incubation samples were diluted with 3 ml of cold PBS, centrifuged and the pellet was washed with the same amount of cold PBS. Final pellet was dissolved in 100 μl of 2 N NaOH and transferred to scintillation vials and counted in beta-counter. The results were expressed as mmoles of carnitine per 1 hour per 1 g of lymphocyte protein. For the assay of the effect of microelements on this process samples were supplemented with Cr^{+3} ions (96.15 μM), selenium Se^{+4} or selenium Se^{-2} (6.33 μM) as well as in combination of selenium with chromium ions.

Fatty acids decomposition was studied in systems contained 20 - 50 μg of lymphocyte protein, Cr^{+3} ions (96.15 μM), selenium Se^{+4} or selenium Se^{-2} (6.33 μM) or combination of selenium and chromium ions. It was 50 pmoles of palmitic acid supplemented with 1 μCi of $[9,10\text{-}^3\text{H}]$ -palmitic acid. Samples were incubated as above and reaction was terminated by precipitation of protein with 200 μl of 10% TCA, than centrifuged and supernatant was collected. After alkalization with 100 μl of 2N NaOH was applied on 0.5 x 2 cm column of Bio-Rad 1X-8, effluent was collected and analyzed using Packard-TriCarb beta-counter. Results were expressed as pmoles of decomposed palmitic acid per 1 hour per 1 mg of protein. In some experiments (50 μM) carnitine was added to the samples.

The results were statistically evaluated using ANOVA multifactorial test.

Results and Discussion

Carnitine is an essential component of mitochondrial system of fatty acids degradation and its transport affects the concentration of this compound in the cell and mito-

chondria, resulting in modulation of concentration of fatty acids, substrates for mitochondrial β -oxidation.

Chromium Cr^{+3} ions, as well as selenium Se^{+4} exhibit low, (16.5% and 22.2%, respectively) stimulatory effect on carnitine uptake by rat lymphocytes, from 13.1 ± 1.27 pmol/min./mg of protein for control, to 15.25 ± 0.65 for Cr^{+3} and 16.01 ± 0.93 for Se^{+4} . Other experimental combinations of both examined ions stimulated this process by 35.5%, to the value above 17 pmoles/min./mg of protein (Table 1).

Table 1. The effect of chromium and selenium ions [Cr^{+3} (96.15 μM), Se^{+4} and Se^{-2} (6,33 μM) on carnitine uptake by rat's lymphocytes (n = 11).

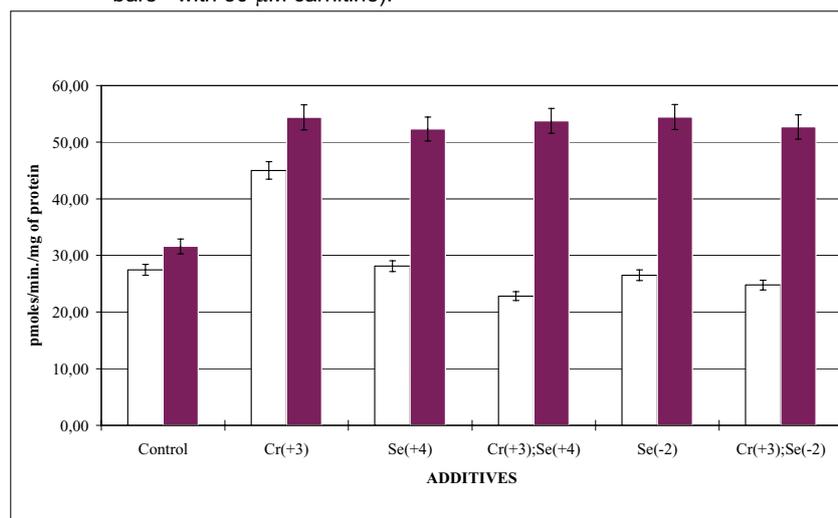
Experimental variant	Carnitine uptake $\mu\text{mole}/\text{hour}/1\text{g}$ of protein
Control	$13,10 \pm 1,27^a$
+ Cr^{+3}	$15,26 \pm 0,65^b$
+ Se^{+4}	$16,01 \pm 0,93^c$
+ Cr^{+3} , + Se^{+4}	$17,77 \pm 0,45^d$
+ Se^{-2}	$17,44 \pm 0,87^d$
+ Cr^{+3} , + Se^{-2}	$17,44 \pm 0,37^d$

Chromium was recognized as an activator of fatty acids degradation (Kuryl et al., 2002, 2004). No effect of selenium (Se^{+4} and Se^{-2}) and slight inhibition of β -oxidation activity observed in the simultaneous presence of chromium and selenium ions needs explanation. It was reported by Combs Jr. (2004), that selenium ions possess the property to bind carnitine. On the other hand, in humans lowering of carnitine level from standard value (40 - 50 $\mu\text{moles}/\text{l}$) to the concentration as low as 10 $\mu\text{moles}/\text{l}$ had no inhibitory effect on β -oxidation activity (data not published). This may suggest different, indirect action of selenium ions on fatty acids degradation.

The results of studies without and with carnitine supplementation are given in Figure 1 and Table 2.

As presented in Fig. 1, carnitine supplementation caused about 15% increase of the activity of fatty acids degradation in lymphocytes of apparently health animals. This may suggest, that in the studied system carnitine is not a limiting factor.

Figure 1. The effect of Cr^{+3} (96.15 μM), Se^{+4} and Se^{-2} (6,33 μM) on β -oxidation activity of fatty acids in rat's lymphocytes before and after *in vitro* supplementation with 50 μM carnitine (open bars - without supplementation, full bars - with 50 μM carnitine).



In both series of supplementation with chromium Cr^{+3} resulted in almost 65% increase of the β -oxidation activity without carnitine supplementation. This effect was very similar to the stimulation of this process in the presence of 50 μM carnitine (70%). In carnitine supplemented cells selenium Se^{+4} or selenium Se^{-2} ions were recognized as good stimulators similar to chromium Cr^{+3} (52.33 ± 3.43 , 54.45 ± 1.95 and 54.41 ± 1.75 pmoles/min./mg of protein, respectively). Simultaneous supplementation with chromium and selenium had no additive effect on the activity of fatty acids β -oxidation in healthy rat lymphocytes. In non-supplemented variants only chromium Cr^{+4} ions exhibits stimulatory effect on the efficiency of fatty acids degradation. Obtained results suggests, that trace elements ions, chromium Cr^{+3} and selenium Se^{+4} and Se^{-2} are involved in carnitine transport across the cell external and internal membranes. The mechanisms of this action must be studied in separate experiments. Statistical evaluation of obtained results by ANOVA multifactorial test indicated statistically significant effect of added chromium and selenium ions and its combinations, as well as of carnitine supplementation, excluding selenium Se^{+4} and Se^{-2} with Cr^{+3} in variant supplemented with 50 μM carnitine (Table 2).

Table 2. The effect of Cr^{+3} (96.15 μM), Se^{+4} and Se^{-2} (6,33 μM) on β -oxidation activity of fatty acids in rat's lymphocytes before and after *in vitro* supplementation with 50 μM carnitine (n = 11).

Media enrichment	β -oxidation activity pmole/min./mg of protein (Mean \pm S.D)	
	without carnitine	with carnitine
Control	27,46 \pm 0,68 ^d	31.60 \pm 0,77 ^a
+ Cr^{+3}	45,02 \pm 1,10 ^e	54,41 \pm 1,75 ^c
+ Se^{+4}	28,11 \pm 1,44 ^d	52,33 \pm 3,43 ^{b,c}
+ Cr^{+3} , + Se^{+4}	22,84 \pm 0,64 ^a	53,77 \pm 3,34 ^{b,c}
+ Se^{-2}	26,51 \pm 0,75 ^c	54,45 \pm 1,95 ^c
+ Cr^{+3} , + Se^{-2}	24,78 \pm 1,46 ^b	52,70 \pm 0,94 ^b

Conclusions

1. Supplementation with carnitine, chromium and selenium, alone or in combinations, activate carnitine transport across extra- and intracellular membranes, resulted in rise of fatty acids degradation.
2. Chromium and selenium ion activate carnitine trans-membrane transport to cells. Selenium Se^{-2} ions were better stimulator of this process than Se^{+4} ions. Simultaneous addition of chromium Cr^{+3} and selenium ions slightly increase carnitine transport in the presence of Se^{+4} but has no effect in the presence of Se^{-2} .

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Summary

The effects chromium Cr^{+3} , selenium Se^{+4} and selenium Se^{-2} ions on carnitine uptake as well as of carnitine supplementation on the activity of β -oxidation in healthy rat lymphocytes were studied. Chromium ions stimulate carnitine uptake and exhibits additive effect on stimulation of this process by selenium Se^{+4} , but not by selenium Se^{-2} . It seems that the mode of action of Se^{+4} and Se^{-2} differs. There is not additional effect of chromium in the presence of Se^{-2} ions. Fatty acids degradation in the absence of exogenous carnitine is stimulated only by Cr^{+3} . Other combinations of ions were ineffective. After carnitine supplementation activation of β -oxidation was observed in all experimental variants.

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The influence of Cr⁺³ picolinate, nicotinate and chloride on apoptosis in human mammary epithelial cells (HBL-100) and breast cancer cells (MCF-7).

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Key words: chromium picolinate, BAX, PARP, ROS, apoptosis

Introduction

Trivalent Cr is recognized as essential trace element necessary for proper insulin function. Dietary Cr⁺³ improve insulin effectiveness by enhancing its binding to receptor and sensitivity of target cells. The requirement of Cr is postulated to be higher in patients with glucose intolerance and diabetes. Individuals suffering diabetes type 2 treated with Cr-picolinate had lower glucose, cholesterol and insulin concentrations in the blood (1). Cr picolinate is second most popular nutritional supplement after calcium, with annual sales over 500 million \$, advertised as a fat burner. However many recent studies demonstrated no effect of Cr(pic)₃ on body composition of healthy subjects. In last decade appeared even claims concerning deleterious action of Cr⁺³ ions. It was noticed that Cr picolinate in ovary cells may cause clastogenic damage. A mutagenic effect of this compound was reported, however in the range of Cr(pic)₃ 0.2÷1.0 mM. DNA fragmentation in macrophage J774A1 cells was also observed (10).

Comparative study of Bagchi et al. (2) using two most popular dietary supplements Cr picolinate and niacin-bound revealed that Cr-picolinate produce much more oxidative damage and DNA fragmentation, when more bioavailable and efficacious niacin-bound Cr compound is not toxic. It suggests that Cr⁺³ safety are largely ligand dependant. Despite of these differences some effects are similar. Balamurugan et al (3) using different Cr⁺³ complexes noticed activation of caspase-3 and poly(ADP-ribose)polymerase (PARP) cleavage in lymphocytes. Pre-treatment of these cells with ROS scavengers attenuates caspase-3 activity suggesting that ROS are upstream activators of this caspase family. It was shown that not only Cr⁺⁶ but also Cr⁺³ compounds cause increases of ROS production. ROS are necessary for optimal tyrosine phosphorylases activities which are key-enzymes in insulin signal transduction (7). It suggests that in case of Cr⁺³, observed ROS increase is relatively small, however these particles might be not only a second messenger of insulin signal transduction but also may play significant role in cell apoptosis mechanism.

The aim of this study was to evaluate the influence of different Cr (III) compounds, including picolinate, on apoptosis induction in mammary epithelial cells.

Material and Methods

Human breast cancer cells line (MCF-7) and human mammary epithelial cells (HBL-100) were obtained from the American Type Culture Collection (Rockville, MD, USA). Cell cultures were maintained in DMEM supplemented with 10% FCS, 50 µg/ml gentamycin, 2.5 µg/ml fungizone, 50 UI/ml penicillin and 50 µg/ml streptomycin in an atmosphere of 5% CO₂ / 95% humidified air at 37° C, and routinely subcultured every 2 or 3 days.

Rabbit anti-PARP, monoclonal mouse anti-Bax, Alexa Fluor 488 chicken anti-mouse secondary antibody, and Alexa Fluor 488 chicken anti-rabbit secondary antibody were also used in this study. All used chrome forms were diluted to the final concentration 10 µg/l of Cr³⁺. Nicotinic acid and picolinic acid were diluted in culture medium, Cr picolinate and nicotinate in DMSO.

Exponentially growing cells were transformed to the 8-chamber culture slides, cultured for 24 h and then incubated in 10% FCS/DMEM for up to 6 h. The control cultures were treated with equivalent concentrations of DMSO suspended in 10% FCS/DMEM. Then cells were fixed in 0.25% formaldehyde for 15 min, washed twice with PBS, suspended in ice-cold 70% methanol and stored in 2°C for 30 min. Then, the samples were stored in -80°C until staining. The cells were washed and incubated for 1h with either primary antibody diluted 1:250. After primary incubation the cells were washed with PBS-1%BSA, and incubated for 1h with 1:500 secondary antibodies. The cells were finally incubated with 5 µg/ml solution of 7-aminoactinomycin D (7-AAD) for 30 min to counter stain the DNA. Then the chamber walls were removed and coverslips were mounted on microscope slides using an anti-fade mounting medium. Probes were analyzed by laser scanning cytometer (LSC) (CompuCyt Corp., Boston, MA, USA). The fluorescence excitation was provided by Argon laser beam. The green fluorescence of Alexa Fluor 488-labeled antibody was measured using a combination of dichroic mirrors and filters transmitting at 520±20nm wavelength and far red fluorescence of 7-AAD transmitting at >650nm. Percent of apoptotic cells (subG1) was measured as a percent of cells with lower concentration of DNA and highest value of red fluorescence in the cell. Another parameter measured was maximal pixel of BAX and PARP fluorescence corresponding to the highest value of fluorescence in the cell, regardless of cellular compartment. The results were statistically evaluated by ANOVA and Tukey's multiple range tests using Prism version 2.0 software. P≤ 0.05 was regarded as significant and p≤0.01 as highly significant.

Results

LSC scans of MCF-7 (cancer cells) and HBL-100 (normal epithelial cells) cells treated with variety forms of chromium revealed an increase in apoptotic cell number (measured by the

percentage of cells in subG1 peak) after exposure to Cr-picolinate. Percent of apoptotic cells was from 5% in control (0 h) to 30% after 6h of treatment in HBL-100 cell line (Fig. 1a) and from 3% in (0 h) to 23% in MCF-7 cells (Fig. 1b). Interestingly other chrome forms don't induce apoptosis in normal and cancer cells. The increase of percent of apoptotic cells in both cell lines after Cr-nicotinate, and nicotinic acid was not statistically significant. Also, after the exposure to picolinic acid cell lines don't show the increase of percentage of cells in sub G1 region. Cells relocated cells from measured areas were showing typical features of apoptosis, i.e. cell shrinkage, chromatin condensation, nuclear pycnosis, and formation of apoptotic bodies. Generally HBL-100 cell line (normal epithelial cells) shows lower aggregation of BAX and PARP then the cancer MCF-7 cell after Cr-picolinate treatment (results not shown). After 6h incubation with Cr-picolinate a significant increase in BAX MP from 6 to 18% in HBL-100 cells (Fig 2a) was observed. After Cr-nicotinate, nicotinic acid and picolinic acid and Cr chloride treatment no significant changes in BAX and 89 kD PARP aggregation was observed in both studied cell lines. During the exposition to Cr-picolinate there was a significant change in 89 kDa fragment of PARP subcellular localization. LSC analysis showed that expression of 89 kDa fragment of PARP (product of caspase 3 and 7 activity) in the nuclear area of the HBL-100 cells increased significantly (Fig.2b).

Discussion

In experimental condition *in vitro* it was observed that chromium picolinate treated human epithelial cells are showing significantly increased participation of cells in sub G1 phase (Fig. 1). During programmed cell death (apoptosis) DNA degradation occur to fragments 50-300 kilo bases. These fragments undergo internucleosomal cleavage and multiplication. Rise of such DNA aggregation is detectable by laser scanning cytometry (LSC) as sub G1 area of DNA histogram and cell number having DNA maximal pixel (4). Such results suggest that this Cr compound might show apoptogenic properties. Such observation is in agreement with results presented by Petit et al. (8), showing implication of Cr⁺³ ions in induction of macrophage apoptosis. They have noticed in human U937 cells treated with Cr⁺³ ions inhibited bcl-2 expression and stimulated bax as well as caspase-3 and -8 expressions. Cr(pic)₃ in contrast to CrCl₃ was found to enhance the rate of appearance of lethal mutations and dominant female sterility.

In our experiment two different lines of mammary epithelial cell were used normal (HBL-100) as well as breast tumor cells (MCF-7). Fact that only Cr picolinate but not Cr nicotinate or chloride possesses such properties suggests importance of ligand in apoptosis induction by chromium (III). This thesis is in agreement with results showing that also picolinic acid (but not nicotinic acid) cause slight increase of cell % in phase sub G1. Apoptosis was quantified by LSC using fluorescence with 7-AAD staining. Per cent of apoptotic cells, in HBL-100 cell

line, increased within 6 h of incubation in medium containing Cr-picolinate (10 µg/l = 192 nM/l) from 5 to 30% and to 12% when picolinic acid was used (Fig. 1). Similar increase but less pronounced was noticed in analogical conditions when neoplastic cells line were used (MCF-7 cells). When CrCl₃ or Cr-nicotinate were used no significant changes were stated in both studied cell lines.

Bcl-2-associated X protein (BAX) is known as strong inducer of apoptosis controlled by transcription factor p53. Bax possesses proapoptotic properties. This protein plays a main role in mitochondrial pathway of apoptosis, together with Bid and VDAC participates in building of omega channels in external mitochondrial membrane. Not only Bax aggregation was observed in used cell lines exposed for Cr-picolinate treatment but also poly(ADP-ribose)polymerase (PARP) activity changes. In spite of fact that poly(ADP-ribose)polymerase repairs damaged DNA and is activated by DNA strand breaks, its proteolytic cleavage 89 kD PARP is closely associated with the apoptosis induction. This cleavage is thought to be a regulatory event for cellular death. Our results indicated increased aggregation of this protein in cells treated with Cr-picolinate (Fig. 2). It suggests that in time of incubation (6h) the increase of cell execution was observed.

In the present study of Berner et al. (5) evaluating chromium tripicolinate safety, the final statement of authors is that this compound is considered safe for intended use up to maximal use level of 2.4 mg Cr picolinate per serving. WHO estimated that 33 µg Cr /d are an adequate dietary daily intake of Cr. It was shown, that very often to reduce insulin resistance and a risk of cardiovascular disease and effects of diabetes type 2, much higher doses of Cr⁺³ were used (up to 1 mg/d). However, long lasting consumption of Cr⁺³ supplements in doses several times higher than recommended may cause negative impact on human organism.

The results of our study indicated that even such low concentration as 10 µg/l of Cr⁺³ [80 µg Cr(picol)₃] may induce apoptosis in normal epithelial and also neoplastic cells. Cr⁺³ nicotinate as well as chloride were not found to show any significant effect on apoptosis stimulation. Obtained results of this *in vitro* study is pointing out the existence of deleterious effect of Cr picolinate.

Summary

Two lines of human epithelial mammary cell were exposed to different Cr⁺³ compounds. Chromium picolinate, most popular Cr nutritional supplement, used for 6 h in concentration of 80 µg/l (192 nM Cr) leads to increased cell death. The rise of % apoptotic cells in sub G1 with increased amount of aggregated DNA was observed in both studied cell lines to be function of time exposure. Significant increase of proapoptotic BAX protein expression was noticed as well as 89 kD PARP. This protein is known as a product of execution phase of

apoptosis. Picolinic acid also was found to increase the DNA damage recognized as increased cell number in sub G1 phase and participation in apoptotic protein expression, however this influence was much lower than shown by chromium picolinate. Chromium nicotinate, as well as chloride, were found not to possess such properties of apoptosis induction.

References

(The literature is available with the author).

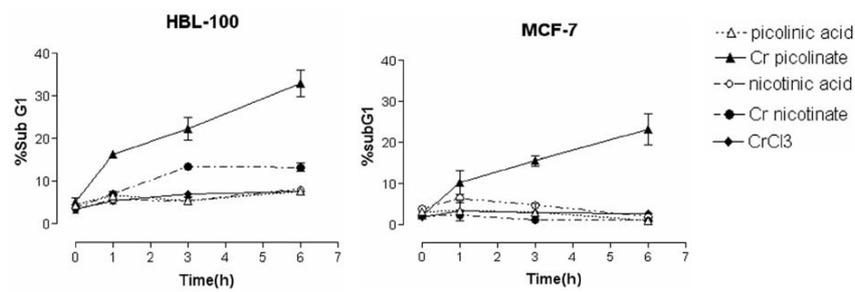


Fig. 1: Percent of apoptotic cells \pm SEM in HBL-100 (a) and MCF-7 (b) cells treated with variety of chrome forms in Cr^{3+} concentration of $10 \mu\text{g/l}$.

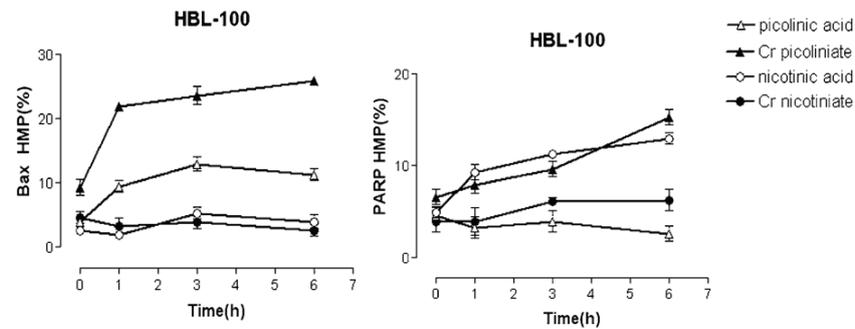


Fig.2: Changes in percentage of BAX HMP (a) and 89 kD PARP-GI (b) in HBL-100 cells after Cr picolinate, picolinic acid, Cr nicotinate, nicotinic acid and Cr chloride treatment.

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Anmerkung:

Die von den Referenten eingesandten Manuskripte wurden im Wortlaut redaktionell nicht bearbeitet, das Layout der Abbildungen und Tabellen wurde weitgehend unverändert übernommen.