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Sven Dänicke
Kirsten Schippel
Ingrid Halle

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Determination of *in vitro* protein synthesis of chick livers using GC-MS analysis of [$^2H_5$]phenylalanine

Sven Dänicke, Kirsten Schippel and Ingrid Halle [1]

Abstract

The GC-MS-system consisting of the auto-sampler A200S (CE Instruments), the GC 8000 (Fisons Instruments) and the quadrupole mass spectrometer MD 800 (Fisons Instruments) was calibrated for measurement of low enrichment of [$^2H_5$]phenylalanine in the range between 0.01 and 1.0 molar percent excess. For this purpose, phenylalanine was converted to phenylethylamine, derivatized to the tertiary-butyldimethylsilyl (t-BDMS) phenylethylamine and peak areas at m/z 183 and m/z 180 were recorded in the selected ion recording mode under electron ionization conditions and subsequently used for isotope ratio analysis.

An *in vitro* study was performed in order to follow the time course of [$^2H_5$]phenylalanine incorporation into the protein fraction of liver homogenates obtained from newly hatched chicks. The incorporation of the label over time increased in a non-linear related manner. Feeding of the breeder hen with a dietary fish oil concentration of 20g×kg^{-1} depressed incorporation of the label in the liver protein fraction of chicks hatched from these hens, and thus decreased protein synthesis, compared to livers obtained from hens fed a diet containing fish oil at 10g×kg^{-1}.

It can be concluded that the tested GC-MS-system is suitable for *in vitro* and *in vivo* experiments on studying protein synthesis using [$^2H_5$]phenylalanine as tracer amino acid.

Keywords: [$^2H_5$]phenylalanine, GC-MS, in vitro protein synthesis, liver, chick

Zusammenfassung

Bestimmung der *in vitro*-Proteinsynthese von Kükenlebern mittels GC-MS-Analyse von [$^2H_5$]Phenylalanin

Das GC-MS-System, bestehend aus dem Autosampler A200S (CE Instruments), dem GC 8000 (Fisons Instruments) sowie dem Quadrupol-Massenspektrometer MD 800 (Fisons Instruments) wurde kalibriert für die Messung einer geringen Anreicherung von [$^2H_5$]Phenylalanin im Bereich zwischen 0.01 und 0.1 Mol-Prozent-Überschuß. Für diesen Zweck wurde Phenylalanin zu Phenylethylamin konvertiert, zum tertiären-Butyldimethylsilyl (t-BDMS)Phenylethylamin derivatisiert und die Peakflächen bei m/z 183 und m/z 180 im "selected ion recording"-Modus unter Elektronenstoß-Ionisation aufgenommen und anschließend für die Analyse der Isotopenverhältnisse verwendet.

Eine *in vitro*-Studie wurde durchgeführt um den Zeitverlauf der Inkorporation von [$^2H_5$]Phenylalanin in die Proteinfraktion von Leberhomogenaten, die von frisch geschlüpften Küken gewonnen wurde, zu verfolgen. Die Inkorporation folgte zeitabhängig einem nicht-linearen Verlauf. Die Fütterung der Zuchthennen mit einer Fischölkonzentration von 20g×kg^{-1} Futter verringerte den Einbau des Markers in die Leberproteinfraktion der Küken, die von diesen Hennen gewonnen wurden, im Vergleich zu denen, die von Hennen, die mit einer Fischölkonzentration von 10g×kg^{-1} Futter gefüttert wurden, erbrütet wurden.

Es kann geschlussfolgert werden, dass das geprüfte GC-MS-System für *in vitro*- und *in vivo*-Experimente zum Studium der Proteinsynthese unter Verwendung von [$^2H_5$]Phenylalanin geeignet ist.

Schlüsselwörter: [$^2H_5$]Phenylalanine, GC-MS, in vitro-Proteinsynthese, Leber, Küken

[1] Dänicke, Sven; Schippel, Kirsten and Halle, Ingrid; Institute of Animal Nutrition of the Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig, Germany

1 Introduction

Measurement of protein synthesis by tracer studies using stable isotopes requires the measurement of very low enrichments in the synthesized protein (usually less than 0.1 atom percent excess). Traditionally, expensive and technically sophisticated isotope ratio mass spectrometers are used for such purposes. However, during the past years it became possible to measure such low enrichments by GC-MS using an isotopic labeled amino acid with a low natural background and a high proportion of heavy atoms within the molecule. [$^2H_5$]phenylalanine was found to be an appropriate candidate for this purpose (Calder et al., 1992; Slater et al., 1995). However, the mentioned authors observed concentration effects on measured fragment ratios which had to be considered in calculation of atom percent excess. Such effects might be, at least in part, instrument specific (Patterson, 1999).

The first aim of the present study was to calibrate our GC-MS-system (consisting of the auto-sampler A200S, CE Instruments, the GC 8000, Fisons Instruments, and the quadrupole mass spectrometer MD 800, Fisons Instruments) for low enrichment of [$^2H_5$]phenylalanine.

The second aim was to follow the time course of incorporation of the label into the protein fraction of a biological system. The liver of the neonatal chick was chosen as such a system because this organ derives nutrients during embryonic development and immediately post hatching exclusively from egg yolk or yolk sac. Moreover, fatty acid profiles of egg yolk can markedly be influenced by dietary fat type (for review see Halle and Jeroch, 1996). As a consequence, lipid metabolism of the liver as the main site of lipogenesis in chickens (Leveille et al., 1975), and consequently also the apo-lipoprotein metabolism might be modified.

2 Material and methods

2.1 Chemicals and reagents

- Diethyl ether (30,996-6),
- sodium citrate (S4641),
- hydrochloric acid (H 7020),
- trichloroacetic acid (TCA, T 9159),
- L-phenylalanine (P 8324),
- L-tyrosine decarboxylase (T 7927),
- pyridoxal 5-phosphate (P 9255),
- acetonitrile (27,071-7),
- N-(tert-BUTYLDIMETHYLSILYL)-N-METHYLTRIFLUOROACETAMIDE (MTBSTFA, B5763) and
- Minimum Essential Medium Eagle (MEM, M 0643)

were all obtained from Sigma-Chemie, Deisenhofen, Germany,

- L-[$^2H_5$]phenylalanine (98 %, lot DLM 1258) was supplied by Promochem (Wesel, Germany).

2.2 Standards

Standards with calculated [$^2H_5$]phenylalanine-molar percent excess (MPE) covering the range between 0.01 and 1.0 MPE were prepared from 60 mM in 0.1 mM HCl phenylalanine (labeled and non-labeled) stock solutions.

2.3 In vitro liver protein synthesis

Generally, the procedure was followed as described by Wang et al. (1998) with some modifications.

A total of 6 one-day-old chicks were obtained from a breeder hen experiment carried out by Halle (2001). Three chicks were hatched from eggs laid by hens fed 10 g fish oil per kg of diet (group 1) and the other 3 chicks originated from hens fed 20 g fish oil per kg of diet (group 2). Chicks were selected from the hatch according to live weight. Chicks of group 1 and 2 weighed 38.4 g and 38.3 g on average, respectively, when the procedure was started. Chicks were killed by cervical dislocation. Livers were quickly dissected, weighed and placed individually in precooled Elvehjem-Potter-vials containing 5 ml of ice-cold MEM (pH 7.4). Then the livers were homogenized by 13 lifts using a tissue-homogenizer. This homogenate was distributed to 4 test tubes in 1 ml portions. Another 4 ml of MEM (pH 7.4) were added to each test tube. Tubes were mixed thoroughly, stopped and then placed into a water bath regulated at 37 °C. After a pre-incubation period of 10 min, 100 µl of a 100 mM [$^2H_5$]phenylalanine in distilled water solution was added to 3 of the 4 test tubes corresponding to each liver homogenate. The tracer containing homogenates were incubated for 15, 30 and 45 min, respectively, with frequent mixing. Metabolic processes in non-incubated and incubated homogenates were stopped by the addition of 5 ml of ice-cold 10 % TCA. Tubes were then centrifuged at 2800*g at 4 °C for 12 min. Supernatants were discarded and pellets were washed with 5 ml of ice-cold 10 % TCA by vortex mixing the tubes in the presence of 3 glass beads. Tubes were centrifuged as described above and this washing procedure was repeated once more to remove any free phenylalanine traces from the protein pellet. After being washed, pellets were transferred to pyrex vials and 4 ml of 6 M HCl were added. Vials were stopped and the sample was hydrolyzed for 24 h at 110 °C. Hydrolysates were filtered using syringe filters, evaporated to dryness under a stream of nitrogen at 40 °C, washed with 2 ml of distilled water and dried again. Samples were finally taken up into 1 ml of 0.1 M HCL.

2.4 Preparation of standards and liver hydrolysates for GC-MS-analysis

Phenylalanine of standards and hydrolysates was converted to phenylethylamine according to Calder et al. (1992). This decarboxylation step improves the chromato-

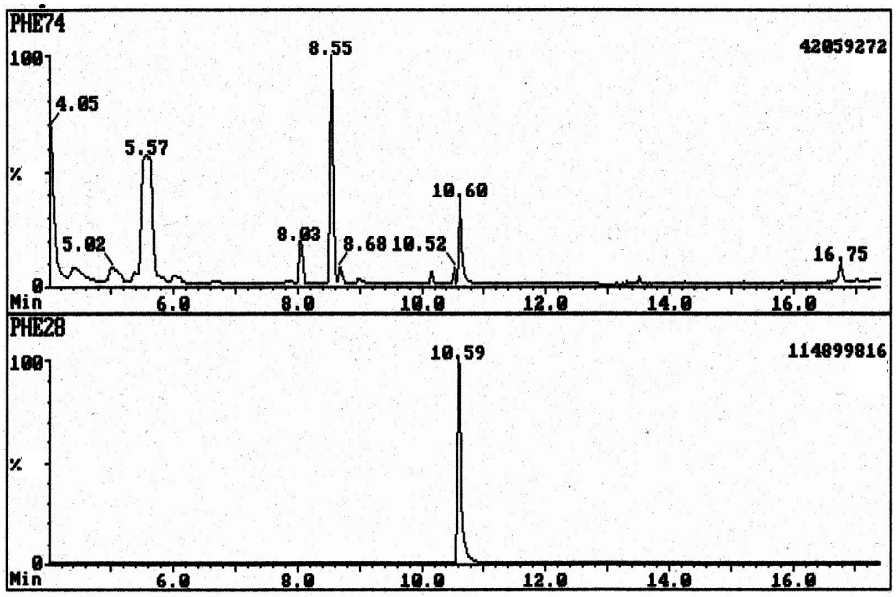


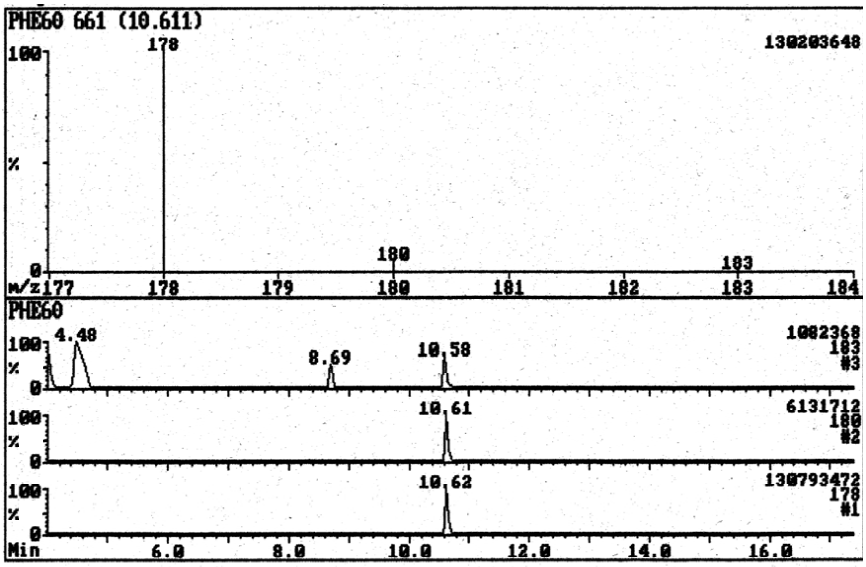
Fig. 1
Total ion chromatogram (a) and single ion monitoring (m/z 178, 180 and 183) (b) of TBDMS[$^2H_5$] phenylethylamine originating from the hydrolysate of the trichloroacetic acid-precipitable fraction of the liver of a one-day-old chick

graphic separation (Calder et al., 1992). Briefly, 15 µl of the standards and 40 µl of the hydrolysates (equivalent to approximately 0.9 nmol phenylalanine) were evaporated to dryness and then dissolved in 200 µL of 0.5 M sodium citrate (pH 6.3). L-tyrosine decarboxylase (0.7 units) and pyridoxal 5- phosphate (0.25 mg) in 100 µL sodium citrate (pH 6.3) were added to the samples and incubated overnight at 50 °C in a water bath. After incubation, 100 µL of 6 M NaOH were added to the samples which were then centrifuged (2800*g, 5 min) after mixing. Supernatants were extracted by 400 µL of diethyl ether and the ether fraction was transferred to another v-vial containing 100 µL of 0.1 M HCl. Phenylethylamine was extracted and dried under a gentle stream of nitrogen at 35 °C. In contrast to the method by Calder et al. (1992), the samples were not converted to the heptafluorobutyric anhydride (HFBA)-derivative since performance of this derivative with our GC-MS-instrument for isotope ratio-analysis was unsatisfactory. Therefore, the tertiary-butyldimethylsilyl (t-BDMS) derivates of phenylethylamine were employed instead (Slater et al., 1995). Dry samples were converted to the t-BDMS[$^2H_5$]phenylethylamine by addition of 200 µL of a 1:1 mixture of MTBSTFA and acetonitrile and heating in sealed tubes for 1 h at 60 °C. The final approximate concentration of phenylethylamine was 4.5 nmol/mL.

2.5 GC-MS analysis of [$^2H_5$]phenylethylamine

Measurements were carried out at a GC-MS system which consisted of the autosampler A200S (CE Instruments) the GC 8000 (Fisons Instruments) and the quadrupole mass spectrometer MD 800 (Fisons Instruments). Derivatized samples were separated on a DB-1 column (J & W Scientific, Folsom, CA, USA) with a length of 15 m, an inner diameter of 0.25 mm and a film thickness of 0.1 µm. Samples of 1 µl volume were injected in the splitless mode. Helium was used as carrier gas. The temperature program of the GC started with an initial temperature of 90 °C for 1 min. Temperature was first ramped to 180 °C at 10 °Cmin^{-1} , held for 2 min, and then ramped to 280 °C at 20 °Cmin^{-1}. Phenylethylamine derivative eluted at 10.6 min (Figure 1). GC-column was diverted to the ion source of the mass spectrometer which was operated under electron ionization conditions. Parameters of the MS were set as follows: source temperature 250 °C, electron energy 70 eV, filament current 4.2 A, trap current 143 µA and source current 1169 µA. The detector multiplier gain was set at 500 V. Isotope ratio analysis was performed as outlined by Slater et al. (1995). In brief, the dominant peak at m/z 178 (Figure 2) is formed from the molecular ion of t-BDMSphenylethylamine at m/z 235. The peak at m/z 183 (m+5) contains all heavy hydrogen. Because of the large difference between peak areas of m/z 178 (m) and m+5 (Figure 2), the m+2 peak (m/z 180) was used for isotope ratio analysis together with the m+5 peak. In this way it is possible to analyze larger sample sizes with low m+5 peaks without an interference caused by an overloading of the much larger peak at m/z 178.

Peaks at m/z 180 and m/z 183 were recorded in the single ion monitoring mode with a span of 0.13 u for both peaks and a dwell time of 0.02 s and 0.44 s, respectively.



(a)

(b)

Fig. 2
Mass spectrum of TBDMS[$^2H_5$]phenylethylamine between m/z 177 to m/z 184 (a) and selected ion monitoring of the ion fragments at m/z 178 (derived from the molecular ion at m/z 235), m/z 183 (m+5) and m/z 180 (m+2) (b). The latter two ion fragments were used for recording of the isotope ratio. Sample was obtained from a hydrolysate of the trichloroacetic acid-precipitable fraction of the liver of a one-day-old chick

2.6 Statistics

Data of the *in vitro* study were evaluated according to a one (live weight, liver weight)- or two(MPE)- factorial design of analysis of variance (ANOVA):

$y_{ij} = m + a_i + e_{ij}$

where y_{ij} = j^{th} replication subjected to fish oil dose i, a_i = fish oil dose (10 or 20 g fish oil×kg^{-1} hen diet), e = error term

$y_{ijk} = m + a_i + b_j + (axb)_{ij} + e_{ijk}$

where y_{ijk} = k^{th} replication subjected to fish oil dose i and incubation time j,
a_i = fish oil dose (10 or 20 g fish oil×kg^{-1} hen diet),
b_j = incubation time (0, 15, 30 and 45 min),
$(axb)_{ij}$ = interactions between a_i and b_j,
e_{ijk} = error term

Simple t-test and test for variance uniformity were applied to evaluate the precision (standard deviation) of GC-MS measurements. Linear regression analysis was performed to generate the calibration curves.

All statistics were carried out using the Statistica for the Windows™ operating system (StatSoft Inc., 1994).

3 Results

3.1 Calibration curve and precision of measurements

Calibration curves between theoretically calculated MPE (x) and measured ratio between m/z 183 and m/z 180 (y) yielded the following linear regressions:

$y = 0.005+0.139*x$
(r^2 = 0.99, for MPE-range from 0.01 to 0.09) (1)

$y = 0.005+0.142*x$
(r^2 = 0.99, for MPE-range from 0.025 to 1.0) (2)

The calibration curve (1) is shown in Figure 3. The calibration curve (2) was generated to cover the MPE-range observed in the *in vitro* study. Both curves gave a similar intercept on ordinate, i.e. the ratio at natural abundance, and comparable slopes, although the slope seemed to increase with higher MPE.

Precision was expressed as standard deviation of measurements (Table 1). Each of the standards used for generation of the calibration curve (1) was measured either 3 times per day one after the other, or once per day three days running. Mean values, standard deviations and coefficients of variation are given in Table 1. The difference between MPE as measured on one day and during three days was not statistically significant for either of the standards. With the exception of the standard with a calculated MPE of 0.07, precision of measurements of the particular standards was homogeneous and consequently not influenced by the order of the measurements (one day,

Table 1
Precision of GC-MS analysis of t-BDMS[$^2H_5$]phenylethylamine

Theoretical MPE	Measured MPE (one day, n=3)			Measured MPE (three days, n=3)			Absolute difference between measured MPE[1]
	Mean value	Standard deviation	Coefficient of variation	Mean value	Standard deviation	Coefficient of variation	
0.01	0.0112	0.0002	2.1	0.0102	0.0010	9.8	0.0010
0.02	0.0218	0.0004	2.0	0.0213	0.0005	2.4	0.0005
0.03	0.0299	0.0017	5.8	0.0284	0.0015	5.4	0.0016
0.04	0.0406	0.0016	3.8	0.0395	0.0009	2.4	0.0011
0.05	0.0524	0.0017	3.3	0.0500	0.0011	2.2	0.0024
0.07	0.0731	0.0004[2]	0.5	0.0713	0.0025[2]	3.5	0.0017
0.09	0.0895	0.0009	1.0	0.0897	0.0008	0.9	0.0003

[1] Differences were not significantly different ($p<0.05$)
[2] Standard deviations were significantly not homogeneous ($p<0.05$)

Table 2
Linearity of GC-MS analysis of t-BDMS[$^2H_5$]phenylethylamine

Theoretical MPE	Measured MPE (different concentrations, n=5)		
	Mean value	Standard deviation	Coefficient of variation
0.01	0.0104	0.0012	11.6
0.02	0.0217	0.0012	5.6
0.03	0.0304	0.0027	9.0
0.04	0.0398	0.0052	13.1
0.05	0.0505	0.0048	9.4
0.07	0.0706	0.0056	8.0
0.09	0.0910	0.0064	7.1

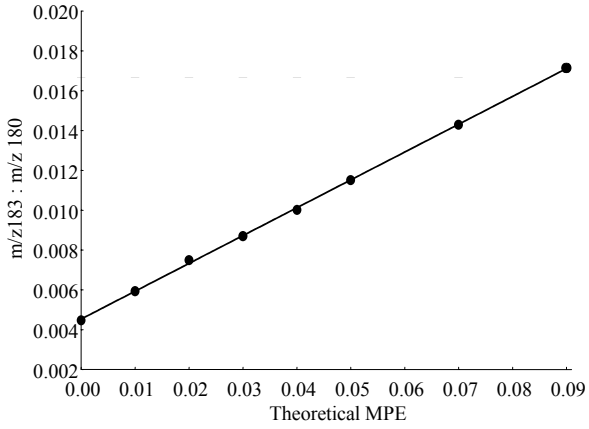


Fig. 3
Relationship between the calculated [$^2H_5$] phenylalanine-molar percent excess (MPE) and the ratio between m/z183 and m/z 180 (calibration curve: $y = 0.005 + 0.139x$, $r^2=0.99$)

Table 3
Summary of linear regression analysis of beam area of m/z 180 (x) on ratio between m/z183 and m/z 180 (y) (m/z183 : m/z 180= a + b·area at m/z 180)

a	b	r^2	Residual standard deviation
0.016	0.00039	0.284	0.0009
0.014	0.00002	0.001	0.0009
0.012	-0.00013	0.053	0.0007
0.010	0.00016	0.091	0.0008
0.009	0.00006	0.049	0.0004
0.008	-0.00004	0.086	0.0002
0.006	-0.00009	0.571	0.0001
0.005	-0.00028*	0.947	0.0001

* $p<0.01$

Table 4
Weight of chicks and their livers
(Chicks were obtained from breeder hens fed diets containing fish oil either at 10 or 20 g×kg^{-1})

g fish oil ×kg^{-1} hen diet	Live weight (g)	Liver weight g	Liver weight g×kg-1 live weight
10	38.4	1.1	29.0
20	38.3	1.1	29.3
Probability	0.811	0.915	0.894
Pooled standard error of means	0.2	0.1	1.6

three days). This means that the system and the derivative are stable over at least 3 days.

3.2 Linearity of measurements

Standards were stepwise diluted with acetonitrile to cover a large range of peak areas in order to study the relationship between peak area (sample size) and the isotope ratio (m+5/m+2). Mean values of these measurements are shown in Table 2 along with the respective standard deviations and coefficients of variation. Generally, coefficients of variation are higher than those observed by constant sample sizes (Table 1) indicating an effect of sample size on measured isotope ratio and consequently on calculated MPE. The relationships between the m+2 peak area and the m+5/m+2 ratio are shown in Figure 4. The respective parameters of linear regression are given in Table 3. Clear concentration effects were observed only for increasing amounts of the standard at natural abundance where a decrease in ratio was observed with increasing sample size.

3.3 In vitro study

Chicks of both groups were selected to have comparable live weights (Table 4). Absolute and relative liver weights were not affected by the feeding regimen of the breeder hen.

Incorporation of labeled phenylalanine into the protein fraction of the livers increased up to 30 min of incubation time and remained at this level for the last 15 min of incubation (significant linear and quadratic effect). This time course was observed for both experimental groups. However, a significantly higher incorporation rate was detected for livers from chicks hatched from hens fed the diet with the lower fish oil content.

4 Discussion

4.1 GC-MS-analysis

Basically, the GC-MS methods described in the present paper are based on the publications of Calder et al. (1992) and Slater et al. (1995) and were adapted to our GC-MS-system, supplied by Fisons Instruments. From their papers and from our preliminary studies on the use of the HFBA-derivative of phenylethylamine, which performed unsatisfactorily at our system, two main problems need to be considered. Firstly, the usefulness of certain derivates of phenylethylamine for isotope ratio analysis seems to be instrument specific. In contrast to the HFBA-derivative, the t-BDMS-derivative was shown to give accurate and reproducible results with our instrument in the enrichment range between 0.01 and 1.0 MPE. Slater et al. (1995) calibrated their VG Masslab Trio-1000 quadrupole mass spectrometer (Fisons Instruments) for the same derivative

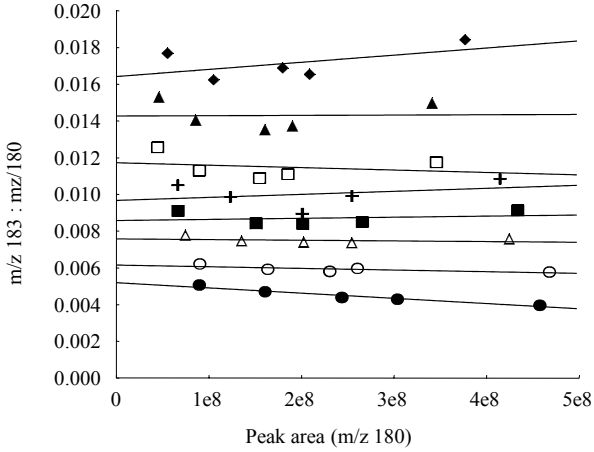


Fig. 4
Effect of sample size (peak area of major beam at m/z 180) on measured ratio between m/z183 and m/z 180

MPE: 0.09, 0.07, 0.05, 0.04, 0.03, 0.02, 0.01, natural abundance.

in the range between 0.005 and 0.12 MPE whereas Calder et al. (1992) reported a range between 0.002 and 0.09 MPE for the HFBA-derivative of phenylethylamine measured at a VG 12-253 quadrupole mass spectrometer. In all cases, the electron ionization mode was run. Secondly, it was emphasized by both mentioned authors that isotope ratio changes with sample size. This was found both for the ratio between m/z 109 and m/z 106 of the HFBA-derivative (Calder et al., 1992) and for the ratio between m/z 183 and m/z 180 of the t-BDMS-derivative as reported by Slater et al. (1995) and observed in the present study. The relationship is dependent on the used derivative, on enrichment and on instrument. Patterson (1999) pointed out that many factors affecting the sensitivity are features of the instrument design beyond the operator's

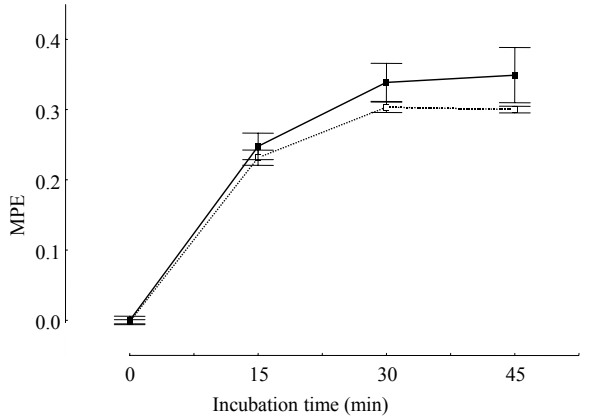


Fig. 5
Effect of incubation time and feeding regimen of the breeder hen (Fish oil at 10 g×kg^{-1} diet and at 20 g×kg^{-1}diet) on [$^2H_5$]phenylalanine-molar percent excess (MPE) in trichloroacetic acid-precipitable fraction of the liver of one-day-old chicks (n=3).

control. There are two possibilities to cope with such concentration effects. Firstly, the concentration dependency might be considered by mathematical description of the relationship between sample size and isotope ratio and a subsequent correction of the life-sample enrichment (Calder et al., 1992; Patterson, 1999). Secondly, sizes of standards and of samples need to be adjusted to each other. The latter approach was applied in the present study to avoid any interference with sample size, although the sample size effects were not obvious for most standards tested for linearity.

4.2 In vitro study

The *in vitro* study can be discussed from a methodological and a biological point of view. Methodologically, the *in vitro* system seemed to work for approximately 30 min as indicated by the time dependent incorporation of the labeled phenylalanine into the protein fraction of the chick livers. During the last 15 min of incubation no change in incorporation was observed. Therefore, the significantly linear and quadratic effects of incubation time (Table 5) result from the initial and final part of the incubation, respectively. The plateau reached at 30 min would indicate that the *in vitro* system was exhausted which could be due to the absence of oxygen supply to the system although *in vitro* measurements of protein synthesis of tissue slices can also suffer from a depletion in oxygen supply (Tischler, 1992). Moreover, cell integrity was disrupted more or less by the homogenization procedure applied which probably had an impact on metabolic control of cell or cell-compartment function. There are a number of other factors which could limit *in vitro* protein synthesis, such as hormone availability and concentration of particular amino acids in the culture medium in relation to the requirement for this amino acid for synthesis of proteins as demonstrated by Hutson et al. (1987) in measurement of albumin synthesis in primary cultures of rat hepatocytes. Alternatively, the saturation in label incorporation observed after 30 min could simply indicate a steady isotopic state, i.e., the rate of incorporation (protein synthesis) equals the rate of release of the label from protein (protein degradation). If it is assumed that free phenylalanine concentration in livers of chicks is approximately 6 µmol×g^{-1} fresh weight (Jahreis and Gruhn, 1978) (approximately 1.2 µmol per incubation) and if further the amount of phenylalanine supplied by the MEM (approximately 1 µmol per incubation) is considered, then the following calculations can be made; the free phenylalanine concentration is raised approximately 4.5-fold by the addition of the labeled phenylalanine (10 µmol per incubation). Therefore, so-called "flooding" conditions were achieved which ensure that enrichment of all possible protein synthesis precursor pools become the same magnitude although this problem was minimized by homogenization of the livers, i.e. intra- and extracellular precursor pools were mixed more or less. Moreover, an increase in phenylalanine concentration in a heart perfusat did not alter the heart protein synthesis rate (McKee et al., 1978). Therefore, protein synthesis rate can be estimated at approximately 29 %×d^{-1} from the present study by taking a mean of 0.24 MPE [$^2H_5$]phenylalanine in protein pool after an incubation period of 15 min and of a mean of 80 MPE [$^2H_5$]phenylalanine in the free pool (calculated from isotope dilution of all 3 sources of free amino acids). This figure is somewhat lower than *in vivo* liver protein synthesis rates (e.g. 70 to 86 %×d^{-1}, Dänicke et al., 2000). Reasons for such discrepancies are not unusual and were discussed elsewhere (Tischler, 1992).

Liver protein metabolism in chicks in relation to fatty acid supply and lipogenesis is interesting in so far as the liver is the main site of lipogenesis in the chicken (Leveille et al., 1975). Consequently, changes in liver lipogenesis could also affect liver apo-lipoprotein synthesis and turnover. It was reviewed by Sessler and Ntambi (1998) that poly-unsaturated fatty acids (PUFA) are thought to suppress expression of genes involved in lipogenesis by binding to a putative binding protein which then binds as a complex to a PUFA-responsive element in the promotor region of PUFA-regulated genes. Moreover, it was outlined by Clarke (1993) in his review that position and number of double bonds within a fatty acid molecule play an important role in modification of fatty acid synthase and other lipogenic enzyme gene expression. Whereas fatty acids of the (n-9)-family do not suppress these enzymes, (n-6) and (n-3)-fatty acids (e.g. linoleic- and

Table 5
Ratio of m/z183 : m/z 180 and estimated MPE in hydrolysates of the trichloroacetic acid-precipitable fraction livers of one-day-old chicks in dependence on hen's feeding and on incubation time

Incubation time (min)	g fish oil ×kg^{-1} hen diet	m/z 183 : m/z 180	MPE
0	...	0.005	-0.001
15	...	0.040	0.240
30	...	0.053	0.321
45	...	0.053	0.325
0	10	0.005	-0.000
15	10	0.041	0.248
30	10	0.055	0.339
45	10	0.056	0.349
0	20	0.005	-0.002
15	20	0.039	0.232
30	20	0.050	0.303
45	20	0.049	0.300
ANOVA (probability)			
Fish oil		0.034	0.034
Incubation time		<0.001	<0.001
Linear		<0.001	<0.001
Quadratic		<0.001	<0.001
Fish oil x incubation time		0.519	0.519
Pooled standard error of means		0.003	0.022

linolenic acid, respectively) are known inhibitors of these genes. In addition, fatty acids within the (n-3)-family having more than 18 carbon-atoms in the molecule (e.g. eicosapentaenic acid and docosahexaenic acid) are much more potent in inhibiting fatty acid synthase. Marine oil which is rich in such fatty acids depresses gene expression more effectively than vegetable oils which do not contain these fatty acids (Clarke, 1993). Therefore, the lower label incorporation into mixed liver proteins of chicks hatched from hens fed the higher fish oil dose as observed in the present study could indicate a suppressed protein synthesis compared to the lower dose.

Taken together, short-time incubation was proved to be useful to demonstrate the incorporation of [$^2H_5$]phenylalanine by using our GC-MS-system into the protein fraction of a protein synthesizing system with a liver homogenate being the model. The nutritional effects observed in the present experiment need to be evaluated by *in vivo* protein synthesis measurements.

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