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Possibilities for reduction of nitrogen (N) excretion from ruminants and the need for further research - a review

Published in: Landbauforschung Völkenrode 56(2006)1-2:19-30

Braunschweig
Federal Agricultural Research Centre (FAL)
2006

Possibilities for reduction of Nitrogen (N) excretion from ruminants and the need for further research – a review

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Abstract

Large proportions of consumed nitrogen are excreted via urine and feces. The excreted amounts vary in ruminants between 60 and 90 % of N-intake depending on animal species, categories and performances.

There exist some possibilities to reduce Nitrogen excretion of ruminant livestock as for instance:

- Avoidance of N-excess in the rations of ruminants or to meet the N-requirements of ruminal microorganisms and the amino acid requirements of the animals.
- Higher animal performances increase N-excretion per animal, but reduce it per unit food of animal origin.
- Shorter growing periods for heifers and longer productive lifespan of cows reduce N-excretion per kg milk.
- To understand the N-metabolism in the rumen and the amino acids metabolism including the splanchnic metabolism (gluconeogenese) in high yielding cows and to take into consideration this knowledge.

Presently there exist in vivo, in sacco and in vitro methods to assess protein degradation and microbial protein synthesis in the rumen. In future in vivo methods will be replaced increasingly by in vitro/in sacco methods calibrated on the base of results of in vivo studies. To harmonize those methods and to improve the knowledge on the splanchnic metabolism is a real challenge for ruminant nutritionists. Such a complicate work expensive in time and money can be only solved in cooperation between various research centres.

Zusammenfassung

Möglichkeiten zur Reduzierung der Stickstoffausscheidungen bei Wiederkäuern und die Notwendigkeit weiterer Forschung – eine Übersicht

Große Anteile des aufgenommenen Stickstoffs werden über Harn und Kot ausgeschieden. Die ausgeschiedenen Mengen variieren je nach Tierart, -kategorie und Leistung bei Wiederkäuern zwischen 60 und 90 % der Stickstoff- (N-) Aufnahme.

Um die N-Ausscheidungen zu reduzieren gibt es einige Möglichkeiten, wie zum Beispiel:

- Vermeidung von N-Überschüssen in den Wiederkäuerrationen bzw. Anpassung der Versorgung an den N-Bedarf der Pansenmikroben und den Aminosäurenbedarf der Tiere.
- Höhere Leistungen steigern die ausgeschiedene N-Menge je Tier, reduzieren sie aber je Einheit Lebensmittel tierischen Ursprungs.
- Kürzere Wachstumsperioden der Färsen und längere Nutzungsdauer der Kühe reduzieren die N-Ausscheidung je kg Milch.
- Die Berücksichtigung der Kenntnisse über den N-Stoffwechsel im Pansen und den Aminosäurenstoffwechsel, einschließlich des Eingeweidestoffwechsels (Glucoseneubildung) hochleistender Milchkühe bei der Fütterung.

Um den Umfang der Abbaubarkeit des Futterproteins und der mikrobiellen Proteinsynthese im Pansen zu ermitteln, existieren derzeit *in vivo*, *in sacco* und *in vitro*-Methoden. In Zukunft werden *in vivo*-Methoden zunehmend durch *in vitro/in sacco*-Methoden ersetzt werden müssen, die *in vivo* kalibriert worden sind. Eine Harmonisierung dieser Methoden und die Erweiterung des Wissens über den Eingeweide-Stoffwechsel ist eine Herausforderung für die Wiederkäuerernährer. Eine derart komplizierte sowie zeit- und kostenaufwändige Arbeit ist nur in Kooperation der verschiedenen Forschungszentren zu bewältigen.

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1 Introduction and objectives

The production of edible protein of animal origin is considered as the main aim of animal husbandry. Foods of animal origin are not essential for human nutrition, but they are extremely helpful to avoid deficiencies in proteins or amino acids as well as important major and trace elements (e.g. Ca, P, Fe, Cu, Zn, Se, I) and vitamins (A, E, B_1, B_2, B_6, B_{12} etc.) esp. in certain groups of population as children, adolescents, pregnant and nursing women. The daily protein yield depends on animal species and categories and their performances. Dairy cows produce the highest protein amounts per day, poultry have the highest protein yield per kg body weight (bw) if we consider high yielding animals (Table 1).

Beside energy and further nutrients sufficient nitrogen in ruminants or essential amino acids in nonruminants are the most important prerequisites for a high protein synthesis in animals. N-excess is mainly excreted via urine and contribute to inefficient N-use, higher feed costs and environmental problems. The processes in the rumen and in the splanchnic tissues are extremely important to understand N-metabolism and to influence/improve N-utilisation by ruminants (Figure 1).

Table 1:
Protein production of various animal species or categories with very high performances (Flachowsky, 2004)

Animal species or category	Performance (per day)	Protein yield (g per day)	Edible protein	
			(g per day)	(g per kg bw)
Dairy cow (650 kg bw)	50 kg milk	1 700	1 615	2.5
Dairy goat (60 kg bw)	5 kg milk	180	170	3.0
Beef cattle (400 kg bw)	1.5 kg body weight gain	285	142	0.4
Fattening pig (80 kg bw)	1 kg body weight gain	150	90	1.1
Laying hen (1.8 kg bw)	60 g egg mass	7.2	6.5	3.9
Fattening chicks (1.5 kg bw)	60 g body weight gain	12	7.2	4.8

bw: body weight

Table 2:
Protein requirements for lactating or fattening ruminants

Reference	GfE	NRC
Dairy cows (2001/2001)		
Maintenance (kg bw)[1)]	450 g uCP (650)[1)]	≈ 1000 g CP (680)[1)]
per kg milk (g fat; g protein per kg milk)[1)]	85 g uCP (40; 35)[1)]	73 g CP (35; 30)[1)]
Dairy goats (2003)		
Maintenance (kg bw)[1)]	100 g uCP (75)[1)]	n.p.
per kg milk (g fat; g protein per kg)[1)]	88 g uCP (40; 35)[1)]	
Fattening bulls (1995/1984) (kg bw; g dwg)[1)]	1000 g CP (350; 1400)[1)]	956 g CP[2)] (350; 1400)[1)]

uCP: utilisable crude protein
n.p.: not present
[1)] Dimensions for figures in brackets
[2)] 2000: 712 g metabolisable protein

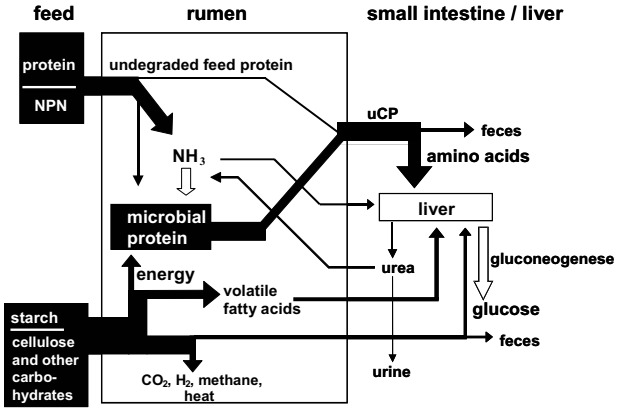

Figure 1:
Energy and N-metabolism in the rumen

In addition to previous reviews (e.g. CAST, 2002; ERM/AB-DLO, 1999; EU, 2002; Walker et al., 2005) the paper deals with N-metabolism in ruminants and contributions to reduce the N-excretion under special consideration of dairy cows.

2 Potential to improve efficiency of dietary N utilization

From the view of animal nutrition N-excretion is defined as following:

N-excretion = N-intake – N-yield in animal products

All nitrogen excreted via urine, feces, skin, sweet etc. could be considered as N-excretion. A certain N-excretion is necessary to maintain the animals. With higher animal performances protein requirement, N-intake and N-excretion per animal increased, but it is reduced per unit of animal product (e.g. milk, meat or other edible proteins, see Table 3). Depending on animal category and performance between 60 and 90 % of consumed N is excreted via urine and feces.

Apart from demand for maintenance nutrient requirements for ruminants consider also the demand for lactation and/or growth/fattening as exemplary shown in Table 2 (see also Schwab et al., 2005).

Table 3:
Production of edible protein of animal origin with different animal species/categories and N-excretion at recommended N supply

Protein source (average bw)	Production per day	Edible fraction %	Protein content in the edible fraction (g per kg fresh substance)	Edible protein		N-excretion	
				g per day	g per kg body weight	kg per kg edible protein	Percentages of intake
Milk							
Cow (650 kg bw)	10 kg	95	34	323	0.5	0.65	75
	20 kg			646	0.9	0.44	70
	40 kg			1292	2.0	0.24	65
Goat (60 kg bw)	2 kg	95	36	68	1.1	0.40	70
	5 kg			170	2.8	0.23	60
Beef (400 kg bw)	500 g bwg[1)]	50	190	48	0.12	2.5	90
	1000 g bwg			95	0.24	1.6	84
	1500 g bwg			143	0.36	1.2	80
Lamb (40 kg bw)	200 g bwg	50	200	20	0.5	1.5	85
	400 g bwg			40	1.0	1.0	80
Pork (80 kg bw)	500 g bwg	60	150	45	0.55	0.8	85
	700 g bwg			63	0.8	0.7	80
	900 g bwg			81	1.0	0.6	75
Poultry meat (1.5 kg bw)	40 g bwg	60	200	4.8	3.2	0.4	70
	60 g bwg			7.2	4.8	0.3	60
Eggs (1.8 kg bw)	50 % lp[2)]	95	120	3.6	2.0	0.6	80
	70 % lp			5.1	2.8	0.35	65
	90 % lp			6.6	3.7	0.2	55

[1)] Body weight gain [2)] Laying performance

2.1 Influences of animal performances

Maintenance of animals requires a certain protein-/N-intake and causes adequate N-excretion in feces and urine. Animals without any performance (only maintenance) fed according to their requirement excrete the same amount of nitrogen as they consume. Table 3 demonstrates the influence of animal performances on protein yield and N-excretion per kg edible protein or as percentages of N-intake. The production of protein via milk, eggs or broiler meat causes lower N-excretion than meat from growing ruminants or pigs. Milk, beef and mutton production are additionally associated with the formation of methane in the rumen. With all types of protein production, N-emissions per kg edible protein decline with increasing yields, but the higher the animal performance the smaller is the decline (Table 3). In a high yielding cow the apparent N digestibility averaged 65 %, with this then partitioned between 34 % excreted in urine and 31 % secreted as milk (Lapierre et al., 2005).

2.2 Age of first calving and productive life

Apart from a high milk yield (Table 3) a first calving in early life and a long productive lifespan of cows can both contribute to make protein production more efficient and to decrease the N-emission (Table 4). The effects of early calving and longer life are more pronounced in low performance cows than in cows with higher milk yields.

2.3 Meet the protein demand by diet composition

To meet the protein demand for ruminants means:
- to meet the N demand for microbes in the rumen and
- to meet the protein/amino acid demand of the animal.

Dietary protein generally refers to crude protein (CP), which is defined for feedstuffs as the nitrogen content x 6.25. The calculated CP content includes both protein and non protein N (NPN, s. Figure 1). NPN in feed, supplements and from the rumino-hepatic cycle are considered to be degraded completely in the rumen; proteins are partially degraded (Figure 1). Ammonia (NH_3) is the most important N-source for microbial protein synthesis. Ruminally synthesized microbial CP (MP) and ruminally undegraded feed CP (UDP) contribute to passage of utilisable (metabolisable) CP (uCP) to the small intestine (Figure 1). The N-metabolism in the rumen is an extremely complex and dynamic process (Nolan and Dobos, 2005; Walker et al., 2005) as shown in Figure 2.

The following termini will be used in the next paragraphs to describe ruminal processes:
- Degradation properties of feedstuffs (degradability and undegradability of protein)
- Passage rate trough the rumen (mainly depending on the level of dry matter (DM) intake)
- Rumen conditions (pH etc.)
- Amount of fermentable energy
- Available N in the rumen (Ruminal N-Balance, RNB)
- Synchronisation of energy and N-supply (N: energy)
- Influence of special nutrients (e.g. fat, sulphur, further minerals).

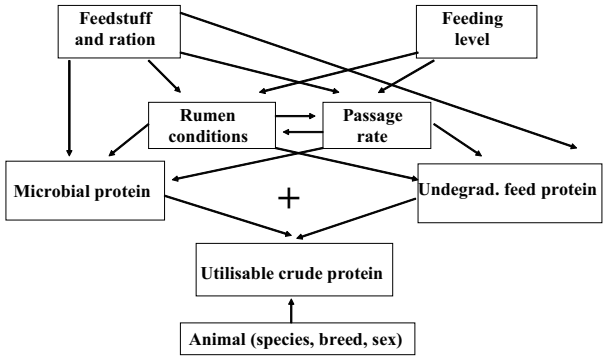

Figure 2:
Factors and interactions influencing the amount of utilisable crude protein (uCP) resp. absorbable amino acids at the duodenum

Table 4:
Influence of milk yield, first calving age of heifers and productive life of cows on N-excretion to produce 1 kg of milk protein[1)] (g per kg protein)

Age of first calving (month)	24			30			36		
Productive life (years)	2	4	6	2	4	6	2	4	6
Milk yield (kg per cow per year)									
4000	700	640	610	750	660	620	860	700	650
8000	510	470	450	540	480	460	590	500	470
12000	450	420	400	460	430	410	490	440	420

1) Body weight: 650 kg; 34 g protein/kg milk; protein yield of slaughtering cows: 25, 12 and 8.5 kg per cow per year for 2, 4 and 6 years productive life (by Flachowsky, 2002)

2.3.1 Microbial protein

The microbial protein synthesized in the rumen is the most important protein or amino acids source for ruminants. Its level depends on the supply of fermented organic matter (Table 5), some other factors like level of feed intake, diet composition, synchronisation of carbohydrate and protein availability in the rumen etc. Those and further influencing factors may be the reasons for the high range of values for microbial crude protein synthesis per kg fermented organic matter (Table 5). N-excess in the rumen and energy deficiency may contribute to N-losses via urine (see Figure 1).

The AFRC (1993) gives an equation to describe the efficiency of microbial crude protein synthesis per MJ fermented metabolisable energy level (gMCP/MJ FME) in dependence on the level of energy intake (L): gMCP/MJ FME = $7.0 + 6.0\ [1\text{-}e^{(-0.35L)}]$

FME = Fermentable metabolisable energy ($ME\text{-}ME_{fat}\text{-}ME_{acids\ in\ silages}$)

L = multiple of energetical maintenance requirement

More details on microbial protein synthesis and influencing factors are described by Flachowsky et al. (2004) and Hristov and Pfeffer (2005) recently.

Table 5:
Amount (g) of microbial protein per kg fermented organic matter in the rumen after various references

Reference	n	Mean	Range
Stern et al. (1994)	n.g.	181	69-266
Lebzien und Voigt (1999)	327	188	63-313
NRC (2001)	n.g.	186	75-338

n.g.: not given

Table 6:
uCP-demand and useful protein degradability in dependence on milk yield (GfE, 2001)

Milk (kg/d)	uCP-demand* (g/d)	Useful protein degradability in the rumen (%)
10	1230	(100)**
20	2050	95
30	2880	80
40	3680	73
50	4480	70

* 650 kg bw and 3.4 % protein in milk
** UDP not required

2.3.2 Undegradable protein (UDP)

The undegradable protein is desfined as the part of feed protein, which is not degraded in the rumen and therefore a source of amino acids in the small intestine. Higher milk yields require relatively more UDP (Figure 3, Table 6) because the microbial protein synthesis is limited by energy supply. Calculation in Table 7 demonstrates the effect of different protein degradabilities on N-excretion for a cow yielding 40 kg milk per day. Nevertheless microbial protein is still the most important protein source for high yielding cows.

Apart from protein sources (soybean meal or corn gluten meal with high UDP) or treatment of protein sources (heat or chemicals like formaldehyde, xylose, tannins) protein degradability in the rumen may also be influenced by the passage rate resp. the retention time in the rumen. Higher passage rate or lower retention time in consequence of a high DM-intake reduce ruminal protein degradability and increases amount of UDP (Figure 4).

Therefore more dynamic models are necessary to assess protein degradation in the rumen. Ruminal protein degradability may be estimated by in vivo, in vitro or/and in situ (sacco) techniques as summarized:

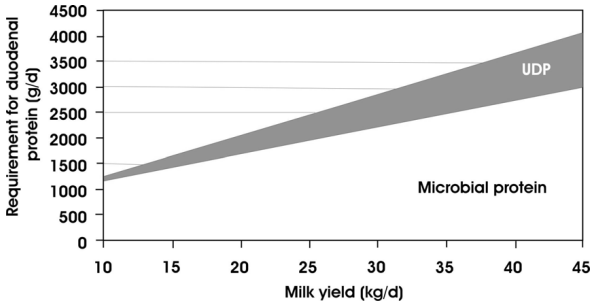

Figure 3:
Fractions of protein at the duodenum

Table 7:
Effect of feedstuffs with different protein degradability on N-excretion (model calculation)

Requirement of a cow with 650 kg BW and 40 kg milk (3.4 % protein, 4.0 % fat):
- 3680 g uCP (MP+UDP, s. Figure 1, Table 6)
- 265 MJ ME

- microbial protein (MP) from ME (10.1 g/MJME)[1)] : 2677 g MP
- required UDP (3680 g – 2677 g) : 1003 g UDP
- CP requirement for 1003 g UDP:
  - 80 % degradability ⇨ 5015 g CP
    N-excess: (5015-3680)g/6.25 = 214 g
  - 73 % degradability ⇨ 3715 g CP
    N-excess: (3715 – 3680)g/6.25 = 6g

[1)] see GfE (2001)

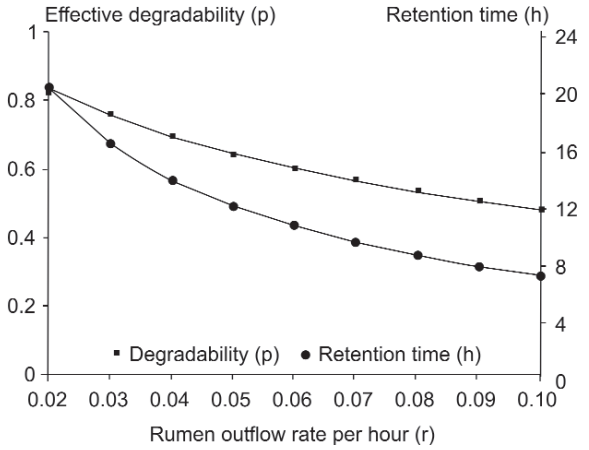

Figure 4:
Effect of rumen outflow rate on degradability and retention time (AFRC, 1993)

In vivo (necessitates duodenally fistulated animals):

1. Regression technique
   (Adding the test protein to a basic ration, additional protein flow at the duodenum = UDP from test protein) assumption: the additional protein does not affect rumen fermentation (max. protein synthesis rates, no interactions)
2. Difference method
   Undegraded feed protein (UDP) = crude protein at the duodenum – (microbial protein + endogenous protein)
   - necessitates a reliable marker for microbial protein and knowledge about the amount of endogenous N

In situ (sacco):

Feedstuff samples in nylon bags incubated in the rumen

- advantage: degradation under ruminal fermentation conditions
- limits:
  - development of a proper microflora in the nylon-bag
  - incomplete removal of microbial protein adhering at feedstuff particles
  - retention time (passage rate) has to be estimated
  - high variability (see Figure 5)

In vitro:

1. Release of NH_3 using rumen fluid
   - limitation: fixation of NH_3 by bacteria, pretending a too low release
2. Solubility in buffers
   - advantage: rumen fluid is not necessary and standardisation is easy
   - disadvantage: correlation to the degradability is low
3. Quantification of crude protein degradation after incubation with rumen fluid
   - limitations: separation of feed and microbial protein is difficult, metabolism end products are concentrated

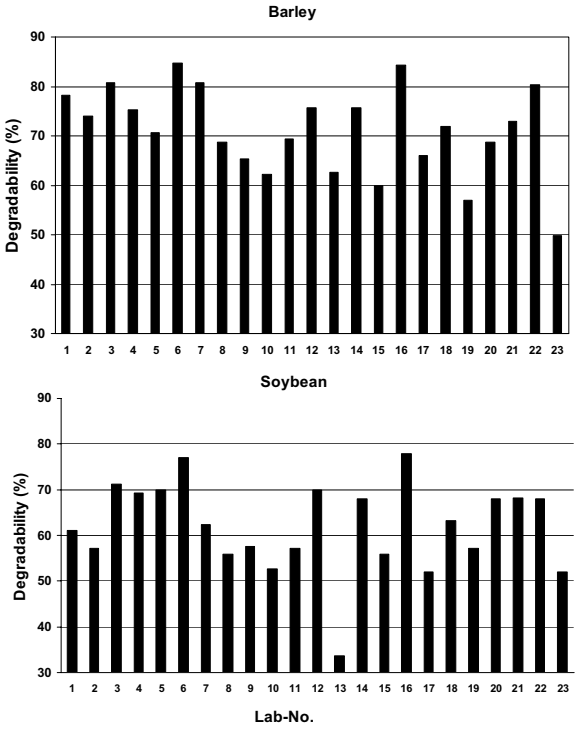

Figure 5:
Protein degradability of barley and soybean meal in nylon bags (Ringtest; Madsen und Hvelplund, 1994)

4. Incubation with proteases: still under testing, but is supposed to have a good potential of standardisation and development

All methods have some advantages and disadvantages. The effective degradability of feed protein obtained from in situ measurements depends on the supposed passage rate. It is higher with lower passage rates and lower with higher ones (see Figure 4). In Europe different passage rates were used (Table 8), showing complications to compare values. Furthermore nylon bag method may influence results of degradation. For example the protein degradation of soybean meal varied between 30 and 80 % if results from 23 laboratories were compared in an European ring test (Figure 5). A critical reflection on the use of the in situ technique was given recently by Huhtanen (2005) and Lebzien and Flachowsky (2005).

To replace the animal studies by in vitro and in sacco methods as well as to harmonize the methods used in Europe seems to be an important task for nutritionists in the future.

2.3.3 Ruminal N-Balance (RNB)

On the one side ammonia in the rumen is extremely important for microbial activities, fibre degradation and microbial protein synthesis; on the other side an excess of ammonia in the rumen may contribute to its absorption, detoxification in the liver and partially to its excretion via

Table 8:
Assumed passage rates (h^{-1}) for calculating the effective degradability of feed protein obtained from nylon-bag experiments in different protein evaluation systems

Country	Supposed passage rate (h^{-1})
France	0.06
The Netherlands	0.06 for concentrates 0.045 for roughages
Scandinavia	0.08
United Kingdom	0.02 – 0.10 depending on feeding level (L, L between 1 and 4.5)

urine (see Figure 1) and possible pollution problems. Therefore the Ruminal N-Balance has been introduced to avoid N-excess during diet calculation. The RNB will be calculated in the following way (GfE, 2001; 2003):

RNB (g/d, g/kg DM or g/MJ ME) =
(CP-uCP) x 0.16 (g/d, g/kg DM or g/MJME)

The RNB should be between 0 and 50 g RNB per day (0-0.3 g N/MJME). Values above 100 g RNB per day need a change in the composition of the diet. Negative RNB-values may decrease feed intake, fibre degradation, microbial protein synthesis and milk yield (Kriete et al., 2004; Riemeier et al., 2004; Lebzien et al., 2006). Apart from the calculation of RNB, urea concentration of milk allows a control of N-supply in the rumen. It should be below 14 mg urea N/100 ml milk (GfE 2001, Table 9). Results in Table 9 demonstrate impressively that N-urine amount increased dramatically with higher RNB in contrast to N-excretion in feces.

2.3.4 Splanchnic metabolism and supplementation with amino acids (AA)

Significant efforts have been made to describe ruminal metabolism in detail (see above). Ruminants need amino acids for maintenance, growth, pregnancy, lactation and further processes. The protein at the duodenum consists of amino acids coming from microbial protein and from UDP. It is extremely difficult to calculate the amino acid pattern of the uCP because of influences of feeding and various proportions of microbial protein and UDP in the protein arriving at the duodenum (Clark et al., 1992; Lebzien, 1997). Furthermore better knowledges are needed for absorption of various AA in the small intestine and the amount of AA used for gluconeogenese. In some cases 25 % or more of glucose from the gluconeogenese (Danfaer, 1999; Lapierre et al., 2005) could be produced from glucoplastic AA (splanchnic metabolism). Lapierre et al.

Table 9:
N-amounts in urine, faeces and milk at different RNBs (Riemeier et al., 2004)

RNB, g/MJ ME	- 0.6	- 0.3	0	+ 0.3
N-urine, g/d	67 ± 0	81 ± 4	153 ± 38	262 ± 115
N-feces, g/d	89 ± 10	96 ± 11	110 ± 12	104 ± 11
Milk urea N, mg/dl	3.2 ± 1.5	4.8 ± 2.8	10.1 ± 2.7	15.2 ± 1.4

(2005) distinguish the amino acids in two major groups: one group undergoes very little hepatic removal and includes the branched-chain amino acids and lysine. For the second group, removal varies between 35 and 50 % of portal absorption, and includes histidine, methionine and phenylalanine. Depending on the milk yield about 400g glucose would be synthesized from AA as shown in Table 10 in a model calculation. Therefore there exist a high variation in AA conversion for milk protein synthesis (Doepel et al., 2004). Descriptions of post-absorptive metabolism assume constant fractional conversions of energy and protein to milk. A quantitative understanding of nutrient metabolism by the post-absorptive tissues is required, and the splanchnic tissues are critical components of the post-absorptive system as they mediate absorption of nutrients and play a role in regulation of metabolite availability (Hanigan, 2005).

Nevertheless many authors investigated effects of an additional supply of rumen protected AA (esp. methionine and lysine) on milk yield, protein synthesis and feed efficiency. Many results did not show any significant effect as summarized by Jochmann et al. (1996), Machle et al. (1999), Robinson et al. (1999) and Wu et al. (1997). For our understanding the prerequisites for a successful supplementation of ruminant diets with amino acids are:

- Knowledge of: · net AA requirement of the ruminants
  - amount and AA composition of microbial protein (MP)

Table 10:
Estimated amount of glucose from amino acids (g/d)

Milk yield (kg/d)	Starch intake (g/d)[2]	Glucose need (g/d)[1]	Glucose from gluconeo-genese (g/d)[2]	Glucose from amino acids (g/d)[3]
10	-	700	700	140
20	1000	1400	1200	240
30	3000	2200	1600	320
40	5000	3000	2000	400
50	7000	3600	2200	440

1) 1.5 x kg lactose/d
2) 20 % from bypass-starch
3) 20 % of gluconeogenese

  - amount and AA composition of undegraded feed protein (UDP)
  - absorption of MP- and UDP-AA
  - utilisation of absorbed AA for protein synthesis
- Protection of AA against degradation in the rumen
- Absorption of protected AA in the duodenum.
- Using of AA for processes apart from milk protein synthesis (e.g. gluconeogenese).

Supplementation of ruminant diets with certain rumen protected amino AA could be a way for better N-utilisation and lower N-excretion, but the knowledges are to low and the results cannot be reproduced in each case presently. Beever and Cottrill (1994) compared some European protein systems and concluded that the principles adopted by all of the protein systems relating to the utilization of absorbed AA are inadequate, and doubts exist about the likelihood of improving this situation until important interactions between individual nutrients are more explicitly represented. Lapierre et al. (2005) conclude in their review, that we must stop using fixed factors of conversion of digestible AA to milk in our predictive schemas and acknowledge that metabolism of AA between delivery from the duodenum and conversion to milk protein will vary with nutrient supply. New information will allow better models to be devised for the prediction of nutrient based responses by the lactating cows. Consideration of biological efficiency, rather than maximal milk yield, will lead to systems, that are economically more sensible for the farmer and that have better environmental impacts.

Finally the AA requirements of ruminants and therefore the success of AA supplementation depend on many influencing factors which are assessed in Table 11. The grade of accuracy to assess the AA requirements is doubtful and bad for many factors. This underlines the present uncertainty.

2.3.5 Further aspects of diet composition

Synchronisation of rumen available protein and energy is considered as one method to increase the efficiency of N-utilisation and to decrease N-excretion by ruminants.

Sinclair et al. (1993) reported that the formulation of diets that are synchronous for energy and nitrogen release in the rumen has shown to increase the efficiency of MP synthesis in the rumen and the so-called synchronisation index (between 0 and 1) has been introduced. In the mean time many studies were done to use the potential for enhancing rumen MP synthesis. The idea is attractive, but the experimental results regarding synchronisation of energy and protein in the rumen have shown considerable discrepancies. In studies from Kaswari (2004, Table 12) synchronisation of energy and protein supply in the rumen in terms of the synchronisation index which was calculated based on in sacco measurements was not correlated to efficiency of microbial protein synthesis. Beside the synchronisation further processes in the rumen (retention time of feeds, inflow of urea etc.) may also influence microbial protein synthesis. The application of feed (e.g. two separate portions per day or total mixed rations, TMR) should have important consequences for rumen fer-

Table 11:
Assessment of amino acid requirements of ruminants

Factors	Calculation	Grade of accuracy
Net requirement		
Milk protein	% protein x milk yield x % AA	good
Maintenance	(UNe + FNe + VN)[1] x 6.25 x % AA	doubtful
Growth	N deposition x 6.25 x % AA	doubtful
Pregnancy	N deposition x 6.25 x % AA	doubtful
AA for gluconeogenesis	(glucose requirement – glucose from bypass-starch) x 0.20	bad
Efficiency of AA-utilization	values from 0.35 - 1.00	bad
Absorption of AA		
Microbial AA	values from 68 - 93 %	quite good
AA from UDP	values from 0 - 100 %	bad
AA flow at the duodenum		
Total AA	from regression x mean AA composition	quite good
from microbes	(63-338 g CP/kg FOM)[2] x AA composition	doubtful
from UDP	(UDP from nylon bag technique) x AA composition	doubtful

[1] see GfE (2001) [2] see Table 5

Table 12:
Synchronization index, MP synthesis, ratio NH_3-N to SCFA and MUN of cows fed different feeding sequences in two trials (Kaswari, 2004)

	1st trial			2nd trial		
Synchronization index	0.76	0.52	0.82	0.85	0.90	0.72
Microbial protein (MP)						
- g/day	1515 ±215	1672 ±398	1576 ±234	1723 ±257	2037 ±183	1777 ±221
- g/kg FOM[1)]	167 ±35	194 ±41	193 ±22	187[b] ±24	257[a] ±40	201[b] ±37
- g/MJ ME	8.8 ±1.3	10.0 ±2.5	9.4 ±1.3	9.4[b] ±1.3	10.6[a] ±1.3	9.4[b] ±1.3
Ratio NH_3-N to SCFA[2)] (mmol/l : mmol/l)	0.12 ±0.11	0.07 ±0.05	0.09 ±0.08	0.14 ±0.09	0.11 ±0.06	0.14 ±0.08

[1)] FOM = fermented org. matter [2)] SCFA = Short chain fatty acids

mentation, but the expected advantage of TMR as compared to separate feeding of concentrates and roughages could not be demonstrated in experiments of Borchert et al. (2006).

An optimal diet composition including all essential major (esp. sulphur) and trace elements as well as vitamins is an essential prerequisite for a high N-utilization in the rumen and to reduce N-excretion.

Recently some non-essential feed addives (e.g. puffering substances, yeasts, microorganisms, NSP-degrading enzymes etc.) are also used in ruminant feeding. Their contributions to reduce N-excretion depends on effects in the rumen and on animal performance. If they increase animal yield or improve feed efficiency (like ionophors in beef cattle), the reduction in N-excretion per animal product may be adequate to higher performances.

2.4 Further measures

In general reduction of N-excretion is a real challenge for genetically modification of crops and animals. Biotech crops with improved amino acid profiles have a real potential to decrease N-excretion significantly, especially in nonruminants as shown by many authors (e.g. Anderson, 1998; Carter et al., 1996). In future, more transgenic plants will be developed with improved capacities to assimilate N into proteins with improved amino acid profiles (Hartnell, 2000), but it is difficult to see advantages for ruminants and the environment under consideration of specific characteristics of ruminants mentioned above.

Production of transgenic livestock provides a method of rapidly introducing “new” gens into animals to improve animal performances and feed use resulting in lower N-excretion per unit food of animal origin. The rate at which genetic improvement technologies are incorporated into production schemes will determine the speed at which the goal is achieved of producing livestock more efficiently with lower nutrient excretion that meet consumer and marked demands (CAST, 2003). Genetical modification of rumen microorganisms could be one step in the direction of lower excretion.

3 Need for rapid and reliable methods for measuring protein degradability and predicting microbial protein synthesis

Processes in the rumen are extremely important for efficient use of nitrogen in ruminants. More details are described in paragraph 2.3. Methods for estimating protein degradability in the rumen as well as their advantages and limits are summarized in paragraph 2.3.2.

There exist a real need to develop rapid and reliable methods for measuring protein degradability in the rumen and predicting microbial protein synthesis.

In future in vivo methods should be replaced by in sacco and/or in vitro methods to assess protein degradability and microbial protein synthesis, but such methods must be calibrated on results of in vivo studies. More experiments are necessary to assess amino acid absorption in the small intestine and the post-absorptive metabolism of amino acids (see Table 11).

The topics mentioned above are a real challenge for allover the world ruminant nutritionists.

4 Tools for monitoring N-adequacy on farms

There exist some tools for monitoring N-adequacy on farms under consideration of the N-metabolism in ruminants as described above:

- Number of ruminants under consideration of species and categories per area
- Intake of feed, N-content of feedstuffs
- N-intake by ruminants (N-input)
- Knowledge of N-requirements of animals and rumen microbes

- Diet calculation (diet composition; Ruminal Nitrogen-Balance, RNB)
- Urea content of milk to assess N-supply of dairy cows
- Protein-/N-content of foods of animal origin
- N-content in manure and N-output
- N-balances on farm level (input/output)

It should be relatively easy from the view of animal nutrition to assess the N-intake of ruminants and to calculate the N-output via foods of animal origin (milk, meat) because of the low range in protein content of milk and meat (see Table 3).

Some authors consider the milk urea Nitrogen (MUN) as a suitable parameter to assess N-supply in lactating ruminants. Breed, pregnancy, body weight, stage of lactation, season, milk yield and composition as well as feed intake and composition of feed may influence the MUN in the milk (see Riemeier et al. 2004). Figure 6 demonstrates the influence of milk yield and lactation stage on the MUN. The MUN depends on the crude protein content of the rations (see Table 9 and Figure 7). But nevertheless there is a certain range in the MUN by the same crude protein content (e.g. between 8 and 20 mg/dl, if 160 g CP/kg DM, see Figure7).

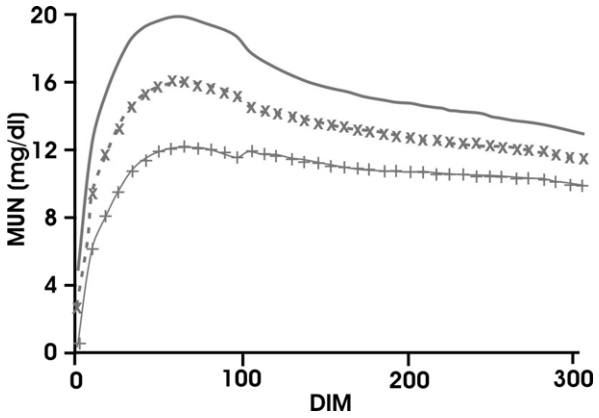

Figure 6:
Predicted milk urea N (MUN; mg/dl) throughout 305 days in milk (DIM) 12000 kg (-), 10000 kg (x) and 8000 kg (+) lactations (Jonker et al., 1998)

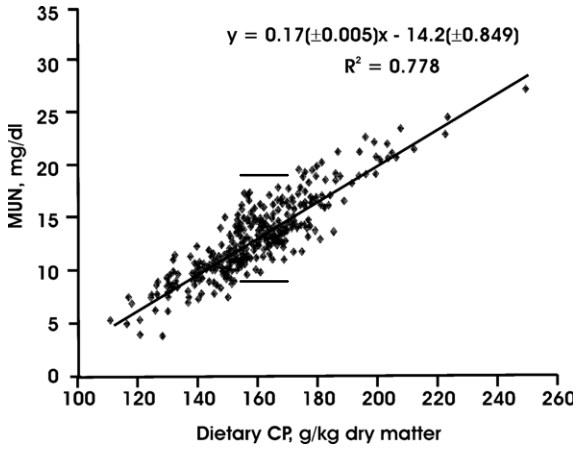

Figure 7:
Association between dietary CP content and MUN for the entire evaluation dataset (n = 306; Nousiainen et al., 2004)

5 Conclusions

Some principles to reduce N-excretion in ruminants are given in Table 13. It is important to distinguish in N-excretion per animal and per unit food of animal origin.

In general N-excess increases N in manure, esp. in urine. The objective of animal nutritionists should be to meet the N-requirements of rumen microorganisms and the AA demand of ruminants depending on animal species, categories and performances to keep the animal healthy and to reduce N in manure. From the view of ruminant nutrition and physiology the following substantial devices are available to reduce N-excretion (see 1-6 in Figure 8):

1. N-content of ruminant diets should consider N-requirements of rumen microorganisms and the AA-demand of animals in dependence on ruminant species and categories as well as animal performances; avoidance of excess N-intake in the diets.
2. Amount of required undegraded feed protein (UDP) depends on animal performances and increases with higher milk yield. Protein sources and passage rate influence UDP-level. Protected proteins (UDP) are adequate, if an increase of microbial protein synthesis (3 in Figure 8) failes to meet uCP requirements (insufficient energy intake).
3. A sufficient and continuous energy and nitrogen supply of the microbes in the rumen are the most important prerequisites for a high microbial fixation on N in the rumen (see Figure 8).

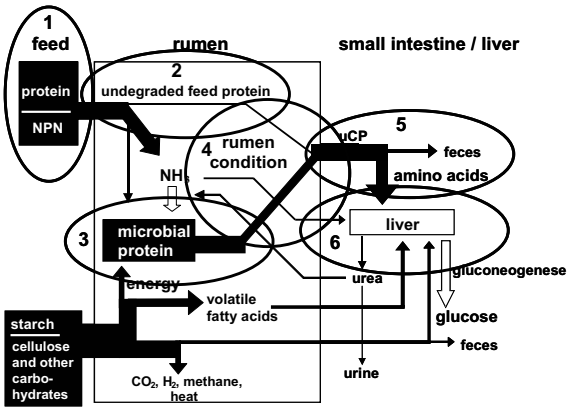

Figure 8:
Substantial adjusting devices of the N-metabolism of ruminants

Table 13:
Principles to influence N-excretion in ruminants

Measure	N-excretion per animal	N-excretion per kg food of animal origin
N-excess in feeding	↑	↑
Increase of animal performance	↑	↓
Shorter growing period for reproduction of cows	↓ ≈	↓
Longer productive life of cows	↑	↓
Positive RNB (>0.3 g N/MJ ME)	↑	↑
High microbial protein synthesis	↓ ≈	↓
Synchronisation of energy and protein degradation in the rumen	≈ ↓	≈ ↓
Rumen protected amino acids (for high performances)	≈ ↓	≈ ↓

↑ Increase ↓ Decrease ≈ No significant influence

4. Optimal rumen conditions (e.g. pH > 6, adequate energy and N-sources, well balanced or light positive Ruminal N-Balance (RNB), meet the requirements of microbes with all essential nutrients etc.); considering of the influence of level of feed intake (passage or rumen outflow rate) on N-metabolism in the rumen and the intestine.
5. Knowledge of amounts, AA composition and AA absorption from microbial (MP) and undegraded feed protein (UDP) are important prerequisites, to understand N-metabolism of ruminants. Protection of AA against degradation in the rumen and absorption of protected AA in the duodenum are parts of this adjusting device.
6. More studies are necessary to understand the metabolism of AA between delivery from the duodenum and conversion to milk protein, probably based on individual AA. This requires more detailed knowledge on the metabolism of splanchnic tissues including the AA utilization for the gluconeogenese in high yielding cows.

For more efficient N utilisation in ruminants and lower N-excretion the following research need can be seen:

- More dynamic considerations of N-metabolism in the rumen (energy and N-intake, microbial protein synthesis, protein degradation depending on feed intake/passage rate etc.).
- Understanding of the amino acid absorption in the intestine, the metabolism of absorbed AA and their conversion to milk (splanchnic metabolism, gluconeogenese etc.).
- Harmonisation of protein evaluation systems in Europe – as a challenge for European ruminant nutritionists in networking.

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