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der Christian-Albrechts-Universität zu Kiel

Experimental Attempts to Improve the Efficiency of Nutrient Utilisation in Ruminants

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Zusammenfassung

Brüsemeister, Frank, 2005 – Experimentelle Ansätze zur Verbesserung der Nährstoffverwertung beim Wiederkäuer

Die einen beträchtlichen Anteil der Kosten der Milchproduktion verursachende Fütterung von Kühen wird maßgeblich durch die Effizienz der Ausnutzung eingesetzter Futtermittel beziehungsweise Nährstoffe beeinflusst – unter letzterem wird hier die Effizienz verstanden, mit der Nährstoffe für die Synthese von Milch und Milchinhaltsstoffen ausgenutzt werden. Aus Kostengründen, aber auch wegen der durch Nährstoffausscheidungen bedingten potenziellen Umweltbelastung, ergibt sich die Notwendigkeit, die Effizienz der Nährstoffausnutzung zu optimieren – das heißt eine möglichst genau auf den Bedarf abgestimmte Nährstoffversorgung der Kühe zu realisieren. Dies bedingt präzise Kenntnisse sowohl des Bedarfs der Tiere als auch der tatsächlichen Versorgung mit entweder in den Vormägen oder im Dünndarm als Hauptorten der Verdauung zur Verfügung stehenden, verdaulichen und absorbierbaren Nährstoffen. Als zentrale Herausforderung der Rationsgestaltung für Milchkühe kann dabei neben der präzisen Bedarfsableitung die Quantifizierung des aus der Pansenfermentation stammenden und für Wiederkäuer essentiellen Mikrobeneiweisses gelten. Mit der vorliegenden Arbeit sollten experimentelle Ansätze zur Verbesserung des Kenntnisstands beider Aspekte verfolgt werden.

Die in dieser Arbeit angewandte Methode – basierend auf der Ausscheidung von Allantoin mit dem Harn –, lieferte semi-quantitative Ergebnisse. Neben der Rangierung waren auch quantitative Unterschiede in der Menge mikrobiell synthetisierten Proteins im Hinblick auf die Rationszusammensetzung plausibel. Eine Ableitung der absolut gebildeten Menge Mikrobenproteins läßt diese Methode derzeit allerdings noch nicht zu. Eine weitere Verbesserung vorausgesetzt, stellt sie aber eine vielversprechende Alternative zu bisherigen Techniken zur Schätzung der mikrobiellen Proteinsynthese dar. Einerseits erfordert sie unter wissenschaftlichen Exaktbedingungen keine fistulierten Tiere und andererseits basiert sie nicht nur auf der chemischen Rationszusammensetzung. Letzterer Ansatz führt häufig zu falschen Ergebnissen, was auch aus einer Studie zur Vorhersagbarkeit der Energiedichte von Getreide-Ganzpflanzensilagen aus deren chemischer Zusammensetzung abgeleitet werden konnte. Das signifikant niedrigere Bestimmtheitsmaß der nur auf der chemischen Zusammensetzung basierenden Vorhersage gegenüber der die Getreideart und den Ort des Anbaus berücksichtigenden Vorhersage der Energiedichte dieser Silagen zeigt deutlich, dass andere Daten als Basis sowohl zur Schätzung der Energiedichte als auch zur Schätzung der mikrobiellen Pro-

teinsynthese nötig sind – zumal beide Aspekte essentiell bei der Optimierung der Rationsgestaltung und mithin der Effizienz der Nährstoffausnutzung sind.

Aus einer stark theoretisch ausgerichteten Studie zu den Zusammenhängen zwischen Methionin- und Cholinstoffwechsel wurde das Konzept eines spezifischen Methylgruppenbedarfs von Milchkühen abgeleitet. Zwar bedarf dieser theoretisch abgeleitete und bisher nur mit einer strukturierten Auswertung publizierter Daten indirekt begründete Ansatz noch der Überprüfung, stellt aber einen wichtigen Beitrag zur Präzisierung des Bedarfs von Milchkühen am Laktationsanfang an der essentiellen Aminosäure Methionin sowie an Methylgruppen dar. Daneben bestätigte die Literaturlauswertung frühere Ergebnisse zur Wirksamkeit der Supplementierung von Rationen für Milchkühe mit pansengeschütztem Cholin am Anfang der Laktation.

Die präsentierten Untersuchungen liefern somit wertvolle Hinweise, sowohl auf Seiten der Nährstoffversorgung als auch des -bedarfs, wie eine Steigerung der Effizienz der Nährstoffausnutzung erreicht werden kann.

Abstract

The overall efficiency of nutrient utilisation of dairy cows is primarily affected by the efficiency with which nutrients are used for milk production. Thus, nutrient use efficiency is a key factor in milk production due to its direct influence on total cost of milk production and its environmental impact. To optimise nutrient utilisation efficiency nutrient supply should match nutrient demand as close as possible. This requires precise knowledge of nutrient requirements and availability to for digestion and absorption in the rumen and the small intestine as the two main sites of digestion. Therefore, quantifying nutrient requirements and rumen microbial crude protein synthesis of dairy cows are two essential tools to improve current feeding systems for dairy cows. This thesis aimed at contributing to this overall objective by enhancing knowledge on both topics from experimental studies.

Urinary allantoin excretion was used as a non-invasive approach to assess rumen microbial crude protein synthesis and delivered a semi-quantitative prediction. Ranking of diets and the magnitude of changes in rumen microbial crude protein synthesis were reflected but the absolute amount was not yet assessable. Provided that future research improves the accuracy of this method, the approach may prove to be a valuable alternative to current methods under research and production conditions because cannulated animals requiring excessive intervention would no further be required. Also, the approach is not based on the chemical composition of diets fed which is only a poor indication of the duodenal flow of microbial nitrogen. This was underlined by the results of the prediction of energy density of whole-crop silage as a feedstuff. The prediction based on type of grain and their origin of growing yielded significant enhancements to the prediction that was solely based on proximate constituents. This observation provides further support that biologically based measures are needed to complement chemical composition to improve predictions of the amount of crude protein that is synthesised by rumen microbes. This would aid in optimising diet composition in order to improve the efficiency of nutrient utilisation. An evaluation of interactions between methionine and choline metabolism of dairy cows provided the basis for a theoretically derived specific requirement for methyl groups. Although this hypothesis, based on the re-evaluation of feeding trials on dairy cows with rumen-protected choline administration, would need experimental verification yet, it may provide a new approach toward a better understanding of methionine requirements at the beginning of the lactation. Moreover, this re-evaluation supported previous results regarding the effectiveness of supplemental rumen-protected choline in diets of dairy cow. The studies reported in this thesis offer attempts to improve the efficiency of

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nutrient utilisation of dairy cows by a better understanding of nutrient requirements and nutrient supply.

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Chapter I

General Introduction

1. General Introduction

Regarding the efficiency of nutrient utilisation of dairy cows the key factor is the efficiency with which nutrients are used for milk production. In most cases, improvements of efficiency can be achieved by an improved supply of limiting nutrients, which consequently will lead to an improved utilisation efficiency of all the other nutrients that are not limiting yield. To utilise nutrients from feedstuffs, they have to be digested and absorbed from either the rumen or the small intestine as the two main sites of digestion in ruminants. Because vast nutrient transformations occur in the rumen, most of the cow's absorbed nutrients are the result of rumen microbial fermentation or modification (Rode, 2000). Therefore, rumen fermentation is a key factor in nutrient utilisation efficiency. Basically, carbohydrates and crude protein from feedstuffs are largely degraded by rumen microorganisms and used for maintenance and growth purposes. Synthesised volatile fatty acids are used as fuel by the ruminant and rumen microorganisms themselves represent the main protein source for the ruminant delivering (crude) protein with a valuable amino acid pattern (Rode and Kung, 1996). Thus, the rumen represents a key site of digestion in the gastrointestinal tract of ruminants, and by maximising fermentation, the cow obtains more volatile fatty acids for energy and more microbial protein (Rode, 2000). However, nutrient utilisation efficiency refers to whole-tract digestibility and absorption, and the small intestine represents the main site of absorption for most nutrients. Thus, improving rumen fermentation only partly means to improve overall nutrient utilisation efficiency: For instance it is commonly accepted that rumen microbial crude protein synthesis at maximum is sufficient to meet requirements for crude protein at a milk yield of about 25 kg per day (Rode, 2000). At higher milk yields, also rumen undegraded crude protein is required to sustain the milk performance level. Hence, dairy cow nutrition includes nutrition of rumen microbes and of the cow itself, which makes dairy cow nutrition a optimisation task between these two objectives although requirements of the dairy cow remains the key criterion for improving the overall nutrient utilisation efficiency.

Because improving the efficiency of nutrient utilisation means to elevate supply of a limiting nutrient, knowledge of supply and requirements is essential. Supply of nutrients available for digestion in and absorption from the small intestine in ruminants is difficult to predict due to the vast ruminal nutrient transformations and the large numbers of factors known to affect rumen fermentation: feeding level and diet composition (Isaacson et al., 1975; Clark et al., 1992; Meng et al., 1999), synchrony between energy and protein supply (Beever, 1993; Casper et al., 1994), energy spilling if crude protein supply is deficient (Van Kessel and Rus-

sell, 1996), and ruminal pH (De Veth and Kolver, 2001). As a consequence, several models for the prediction of the passage of crude protein and amino acids to the duodenum exist – frequently delivering varying and conflicting results (Bateman et al., 2001a, b). Thus, requirements have not been entirely investigated and quantified now. As an example, a limiting factor in most dairy cow diets besides energy supply is the first limiting amino acid. Amino acids that have been considered as being limiting are histidine, isoleucine, leucine, lysine and methionine (Pisulewski et al., 1996; Schwab, 1992 and 1996; Robinson et al., 1999; Vanhatalo et al., 1999; Varvikko et al., 1999).

In order to optimise nutrient utilisation efficiency in dairy cow nutrition, precise knowledge on both, supply and requirements, is necessary. The three studies reported in this thesis follow different approaches and pathways that may lead to improvements of the efficiency of nutrient utilisation. In the first experiment the adequacy of urinary allantoin excretion as a non-invasive marker for predicting ruminal microbial crude protein synthesis is investigated and diet effects on the efficiency of ruminal microbial crude protein synthesis are considered. The third study dealt with a similar issue but is more oriented on feedstuff characteristics and their influence on energy density. Both studies focus on improving nutrient utilisation efficiency by optimisation of rumen fermentation. The second study aims at improving the predictability of the nutritional status of the dairy cow. Interactions between methionine and choline metabolisms were investigated. This approach is a contribution toward improving nutrient utilisation efficiency by a better understanding of methionine requirements.

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Chapter II

Effects of amount of intake and stage of forage maturity on urinary allantoin excretion and estimated microbial crude protein synthesis in the rumen of steers

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Summary - Using ruminally cannulated steers, we investigated how urinary allantoin excretion was related to variations in feed intake and stage of forage maturity. Further, different approaches were compared for predicting ruminal microbial crude protein (MCP) synthesis and its efficiency. Experimental diets were arranged in a replicated 3 x 3 Latin square design (Experiment 1) and a 4 x 4 Latin square design (Experiment 2). In Experiment 1, a mixed diet (forage to concentrate, 68:32 on a dry matter (DM) basis) was fed at three intake levels corresponding to 1, 1.5 and 2 times maintenance energy requirements. In Experiment 2, four silage-based diets were fed based on perennial ryegrass (*Lolium perenne* L.) which was harvested at four maturity stages. Both experiments demonstrated the influence of diet on microbial growth rate and by this on efficiency of MCP synthesis, although the magnitude of the effects differed between approaches used for estimating MCP. Linear functions satisfactorily characterised the relationship between urinary allantoin excretion (y) and digestible OM intake (x, kg/day; Experiment 1: $y = 7.94 + 17.34x$; $R^2 = 0.785$) or intake of OM effectively degraded in the rumen (x, kg/day; Experiment 2; $y = 22.32 + 5.93x$; $R^2 = 0.695$). Urinary excretion of allantoin permitted a semi-quantitative prediction of MCP synthesis: Ranking of diets and magnitude of changes in MCP synthesis were reflected.

1. Introduction

In ruminant nutrition, maximum efficiency of utilisation of nutrients requires precise knowledge on transformations of nutrients in the rumen as rumen microbial protein contributes a significant proportion to the protein reaching the small intestine. Several approaches for estimating ruminal microbial crude protein (MCP) synthesis do exist and each has its advantages and disadvantages. In general the chemical composition of forages is a poor indication of the duodenal flow of microbial N in ruminants fed all-forage diets (Gosselink et al., 2003). The yield of ruminal MCP not only depends on the solubility of dietary CP but also on supply of fermentable energy sources and degree of ruminal synchronisation of CP and carbohydrate catabolism (Beever, 1993). This makes yield of MCP difficult to predict. Attempts to quantify MCP yield using abomasally or duodenally cannulated animals are time and labour consuming and require excessive intervention. Non-invasive methods controlling efficiency of MCP production *in vivo* have the advantage of being applicable under research and production conditions. Purine derivatives are a potential marker of MCP synthesis in the rumen (Stangassinger et al., 1995). Digesta reaching the small intestine for subsequent digestion and nutrient absorption contains considerable quantities of nucleic acids nearly solely originating from MCP (Calsamiglia et al., 1996; Djouvinov und Todorov, 1996). Microbial nucleic acids are highly digestible (Chen et al., 1990a). In contrast to sheep, purine bases from degraded nucleic acids are rapidly and virtually completely oxidised within the intestinal mucosa of cattle (Chen et al., 1990b; Verbič et al., 1990) and then mainly excreted in urine (Verbič et al., 1990). For cattle, allantoin is the most predominant purine derivative (see Shingfield, 2000). Based on a comprehensive review, Shingfield (2000) concluded that urinary excretion of purine derivatives only does allow a ranking of diets in terms of fermentability but no accurate prediction of MCP synthesis.

The objectives of this study were (I) to compare the adequacy of different approaches to predict ruminal MCP synthesis based on a non-invasive experimental method, i.e., urinary allantoin excretion, and (II) to investigate diet effects on efficiency of ruminal MCP synthesis. Therefore, two experiments were conducted differing in amount of intake (Experiment 1) or in amount of intake and stage of forage maturity and thus, chemical composition (Experiment 2).

2. Materials and methods

Two experiments with six (Experiment 1) and four (Experiment 2) adult steers were conducted. Animals were housed indoors in individual tie stalls in a temperature controlled room (18 °C) under continuous lighting. The daily allotment of feed was offered in two equal meals at 07:00 and 19:00 hours. Prior to feeding, all dietary ingredients were completely mixed by hand. The steers had continuous access to water.

Experiment 1

Experiment 1 formed part of a larger experiment on effects of intake level on ruminal particle size distribution and large particle breakdown (Kovács et al., 1997). Briefly, five ruminally cannulated Angler Rotvieh steers, ranging in body weight from 780 to 890 kg and one ruminally cannulated Hinterwälder steer weighing 600 kg were used. The steers received a mixed diet consisting on an average of (g/kg of dry matter (DM)) 430 Italian ryegrass, 250 maize silage, 300 concentrate and 20 mineral-vitamin mix. The chemical composition of the diet is reported in Table 1. Tabulated values (Universität Hohenheim – Dokumentationsstelle, 1991) were used to estimate metabolisable energy concentrations of dietary ingredients. Three intake levels were chosen to represent 1.0, 1.5 and 2 times estimated energy maintenance requirements according to ARC (1980) and hereafter referred to as low, medium and high intakes. Treatments were arranged in a 6 animals × 3 periods repeated Latin square design (Gill, 1978). Experimental periods lasted 21 days, allowing 7 days for adaptation to the intake level and 14 days for sample collection.

Table 1: Dry matter content (%) and chemical composition of a mixed diet fed to steers at three amounts of intake (Experiment 1) and of silages of perennial ryegrass cut at four different harvest dates (Experiment 2)

	Experiment 1		Experiment 2			
	Mixed Diet	8 May	22 May	7 June	19 June	
Dry matter (DM)	44.2	20.1	22.6	23.9	18.3	
Composition of the DM (%)						
Ash	8.8	9.6	7.9	7.0	6.4	
Acid ether extract ²	3.7	4.6	3.4	2.7	2.8	
Crude protein	13.4	16.0	11.3	8.7	7.7	
Crude fibre	19.8	19.8	24.5	28.2	31.8	
Acid detergent fibre	24.1	22.7	26.9	32.2	36.7	
Neutral detergent fibre	45.1	35.9	45.4	55.3	62.6	
Acid detergent lignin	2.3	1.3	1.7	2.8	3.8	

¹Each value represents the average of at least 6 replicates

²Acid hydrolysis prior to extraction

Experiment 2

Details of this experiment related to ruminal bacterial fractions have been reported by Philipczyk et al. (1996). Four ruminally cannulated Angler Rotvieh steers (average body weight 650 kg \pm 28 kg) were fed on first-cut perennial ryegrass (*Lolium perenne* L., variety Gremie) silages. The grass was harvested at four different stages of maturity during primary spring growth in 1991: late shooting (8 May), early to mid heading (22 May), late heading (7 June) and early flowering (19 June). The grasses were cut with a rotary mower in the morning and ensiled after a short (< 3 h) wilting period. During ensilaging, formic acid (85%) was added at a rate of 2.3 l/t of grass. Silages were stored a minimum of 150 days prior to the start of the feeding trials. The chemical composition of the silages is given in Table 1. All four silages were supplemented with 0.49 kg DM of a premix containing barley, a mineral-vitamin premix and the digestibility marker titanium(IV)-oxide (TiO₂) in the ratio of 76:19:5. Silages from 22 May, 7 June and 19 June were balanced with soybean meal to approximately 13% CP in the dietary DM. The ratios (g/kg DM) of silage:soybean meal:premix were 944:0:56, 908:45:47, 840:107:53, and 799:140:61 for the four diets containing silages from 8 May, 22 May, 7 June and 19 June, respectively. Voluntary intake of the diets was determined during a 13-day period before the start of the collection periods. During the collection periods, feed intake of each steer was restricted to 85% of its previously recorded voluntary feed consumption.

Each diet was offered to each animal using a 4 \times 4 Latin square design with 14-day periods. The diets containing the four different silages were designed according to the respective harvest date of the perennial ryegrass.

Sample collection

In both experiments representative samples were obtained from each dietary ingredient, composed by period and stored (silage at -20 °C) for subsequent analyses. Feed refusals, if any, were collected twice daily, stored under vacuum during the trials, and composed by animal over the collection period. Total collection of urine was made with the aid of harnesses connected to containers containing 100 ml of H₂SO₄ (10% v/v) during two consecutive days (days 8 - 9 and days 13 - 14 of the sampling periods in Experiment 1 and 2, respectively). The urine was weighed and sampled every 4 hour. If necessary, additional H₂SO₄ was added to maintain the pH of the urine below 3.0, and subsamples of the acidified urine were kept at -20 °C until analysed. In Experiment 1 total faecal collections, being started and finished at 07:00 hours, were made over 5 days. Faeces were collected in plastic pans. Wet faeces were weighed twice daily to the nearest 10 g and mixed, and an aliquot of 5% was

transferred to an accumulative sample container and immediately stored at $-20\text{ }^{\circ}\text{C}$. In Experiment 2, faeces were spot-sampled twice daily over the entire 14-day collection period around feeding times directly after defecation or after rectal stimulation. The sample was mixed, 200 g were transferred to an accumulative sample container and stored at $-20\text{ }^{\circ}\text{C}$.

Analytical procedures

The DM of silages, feed refusals and faeces was estimated by freeze-drying and subsequent oven-drying at $105\text{ }^{\circ}\text{C}$ overnight. For all other analyses freeze-dried silages, feed refusals and faeces, and air-dried concentrates and premixes were used. Proximate constituents and detergent fibre fractions of all materials in both experiments were determined according to official German methods (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten; Bassler, 1976). In experiment 2, TiO_2 was determined photometrically in a Kjeldahl-digested TiO_2 -solution (40 mg/l TiO_2 in 1.3 M H_2SO_4) and in Kjeldahl-digested samples of faeces (Brandt and Allam 1987). Thawed samples of urine were neutralised with NaOH and diluted 1:20 with double-distilled water. Allantoin in urine was analysed by reversed-phase-HPLC (Roskopf et al. 1991) with 0.005 M $\text{NH}_4\text{H}_2\text{PO}_4$ as eluant (Giesecke et al., 1993).

Calculations and statistical methods

Whole-tract organic matter (OM) digestibility was estimated from the difference of daily OM intake and daily faecal OM excretion (Experiment 1). In Experiment 2, OM digestibility was calculated from the concentrations of TiO_2 in feed and faecal DM. These values were then used to calculate the digestible OM intake (DOMI). The quantity of OM truly digested in the rumen (TDOMR, kg/day) was obtained by multiplying the intake of digestible OM by the factor 0.55 assuming that digestion occurring in the rumen was 55% of OM intake (Stern et al., 1994). The quantity of OM apparently digested in the rumen (ADOMR, kg/day) was obtained by multiplying the intake of digestible OM by the factor 0.6, i. e. the proportion of whole-tract OM digestion occurring in the rumen. This value was the mean derived from experiments with dairy cows fed on a total of ten mixed rations in earlier studies (Schröder et al., 1989; Südekum et al., 1992, 1994; Schröder et al., 1997). This value is only slightly lower than the factor 0.64 reported by Rohr et al. (1986) and was chosen because general conditions under which the cows were kept in the earlier studies were similar to those used for the steers in the present study. Extent and rate of OM degradation and the effective degradability of OM in the rumen (EDOMR) of the silages in Experiment 2 were calculated from *in sacco* incubations of the silages in the rumen (Schäfer, 1996). The EDOMR values were calculated according to McDonald (1981), assuming a rumen solid outflow rate of 5%/h as suggested by

the ARC (1984) for medium intake levels. To derive the EDOMR values for the mixed rations, EDOMR values of barley in the premix and of soybean meal were assumed to be 80% and 70%, respectively.

Ruminal MCP synthesis was estimated using three different approaches. The first approach was that of Chen and Ørskov (2003) with additional assumptions that the N content of MCP is about 16% and urinary excretion of allantoin by cattle is about 88% of total purine derivative excretion. The latter figure represents the average of values published in the literature (see Shingfield, 2000; Orellana Boero et al., 2001; Gonzáles-Ronquillo et al., 2004):

$$\text{MCP}_{\text{ALL-CØ}} \text{ (g/day)} = 0.727 \times 6.25 \times [\text{Allantoin (mmol/d)}/0.88 - 0.385 \text{ (mmol/d)}] \times \text{BW}^{0.75} / 0.85$$

where $\text{BW}^{0.75}$ is metabolic body weight (kg) and the term $(0.385 \times \text{BW}^{0.75})$ denotes endogenous urinary allantoin excretion (Verbič et al., 1990).

Second, an approach based on the estimated exogenous urinary allantoin excretion alone as proposed by Ehrentreich (1992) was used:

$$\text{MCP}_{\text{ALL-E}} \text{ (g/day)} = 131.7 \times \text{exogenous urinary allantoin-N (g/day)}$$

The estimate of endogenous urinary allantoin was based also on the data of Verbič et al. (1990).

The third way to derive a figure for MCP synthesis was based on the generalised assumption that 184 g MCP were synthesised per kilogram of ADOMR (Czerkawski, 1986). This concept was chosen as an alternative estimation since it is not based on the amount of excreted purine derivatives; the estimate was denoted as $\text{MCP}_{\text{ADOMR}}$.

Data of Experiment 1 were analysed using the general linear models (GLM) procedure of SAS (1988) considering treatment (intake level), animal and period as main effects. Treatment means were separated further with the following orthogonal contrasts: low intake versus high intake and medium intake versus (low intake + high intake). Significance was declared at $P < 0.05$ unless otherwise indicated. The statistical model for data of Experiment 2 included treatment (stage of forage maturity, i.e., harvest date of perennial ryegrass), animal and period as main effects. Treatment means were analysed further with the following orthogonal contrasts: May versus June (silages from 8 and 22 May versus silages from 7 and 19 June); May (silage from 8 May versus silage from 22 May); and June (silage from 7 June versus silage from 19 June).

A linear regression was used to describe the allantoin excretion per day with DOMI (kg/day) as independent variable in experiment 1:

$$\text{Allantoin (mmol/day)} = a \text{ DOMI} + b$$

In experiment 2, EDOMR was used as independent variable:

$$\text{Allantoin (mmol/day)} = a \text{ EDOMR} + b$$

3. Results

Experiment 1

Intake and digestibility of OM and urinary allantoin excretion by steers in Experiment 1 are reported in Table 2. The daily OM intake ranged from 5.7 kg at the low intake to 11.3 kg at the high intake. The digestibility of OM decreased from 83.7 to 78.4% as intake increased; this was caused most likely by a higher passage rate as time of fermentation in the rumen was shortened. Although there were pronounced differences in daily total urinary allantoin excretion, the excretion per unit (kg) of DOMI was similar for all levels of intake, reflecting a strong relationship between DOMI and total allantoin excretion in Experiment 1. If the endogenous contribution was taken into account, the differences between treatments of urinary excretion of allantoin from exogenous origins related to DOMI (kg) were not significant either (data not shown).

Table 2: Intake and digestibility of organic matter (OM) and total urinary allantoin excretion of steers fed mixed diets at three amounts of intake (Experiment 1)

Intake (kg/d)	Intake level			Effect (P) of intake	Contrasts (P)		SEM ¹
	Low	Medium	High		Low vs. High	Medium vs. Others	
OM	5.7	8.3	11.3				0.2
DOMI ²	4.7	6.7	8.9	<0.001	<0.001	0.304	0.2
ADOMR ³	2.8	4.0	5.3	<0.001	<0.001	0.611	0.1
TDOMR ⁴	3.1	4.5	6.2	<0.001	<0.001	0.310	0.1
Digestibility ⁵ (%)	83.7	81.0	78.3	0.009	0.003	1.000	0.9
Allantoin excretion (mmol/day)	90	127	160	0.002	<0.001	0.878	8.6
(mmol/kg DOMI)	19.1	19.0	17.9	0.749	0.501	0.756	1.2

¹Standard error of least squares means

²Digestible OM intake

³OM apparently digested in the rumen; it was assumed that 60% of whole-tract OM digestion occurred in the forestomachs (Schröder et al., 1989; Südekum et al., 1992, 1994; Schröder et al., 1997)

⁴OM truly digested in the rumen; it was assumed that digestion occurring in the rumen was 55% of OM intake (STERN et al., 1994)

⁵OM apparently digested in the whole tract

Table 3 shows the estimated amounts of MCP as well as the efficiency of MCP synthesis in Experiment 1. The amount of MCP increased with increasing feed intakes regardless of the approach used for calculating MCP synthesis and this general observation was supported by statistical evaluation of the data (contrasts between treatment means). Moreover, the numerical differences between diets were similar for estimates based on the $MCP_{ALL-CØ}$, MCP_{ALL-E} and MCP_{ADOMR} approaches. However, the efficiency of MCP synthesis showed a distinctly different picture. Although statistical evaluation of data would indicate significant diet effects for values based on MCP_{ADOMR} , the differences were very small numerically, indicating that efficiency was similar across feed intake levels. With the two other estimates, the efficiency of MCP synthesis only tended ($P < 0.15$) to be higher for the high than the low intake level.

Table 3: Amounts (g/day) and efficiencies of rumen microbial crude protein (MCP) synthesis in steers fed a mixed diet at three amounts of intake, estimated on the basis of urinary allantoin excretion or organic matter apparently digested in the rumen (Experiment 1)

	Intake level			Effect (<i>P</i>) of intake	Contrasts (<i>P</i>)			SEM ¹
	Low	Medium	High		Low vs. High	Medium vs. Others	Others	
MCP² (g/day)								
MCP _{ALL-CØ}	245	460	656	0.002	<0.001	0.885	53.8	
MCP _{ALL-E}	295	556	794	0.002	<0.001	0.885	65.3	
MCP _{ADOMR}	523	742	982	<0.001	<0.001	0.618	17.4	
Efficiency of MCP synthesis (g N/kg TDOMR³)								
MCP _{ALL-CØ}	13	16	17	0.241	0.133	0.451	1.7	
MCP _{ALL-E}	15	20	20	0.234	0.129	0.449	2.1	
MCP _{ADOMR}	27	26	25	0.004	0.001	0.784	0.2	

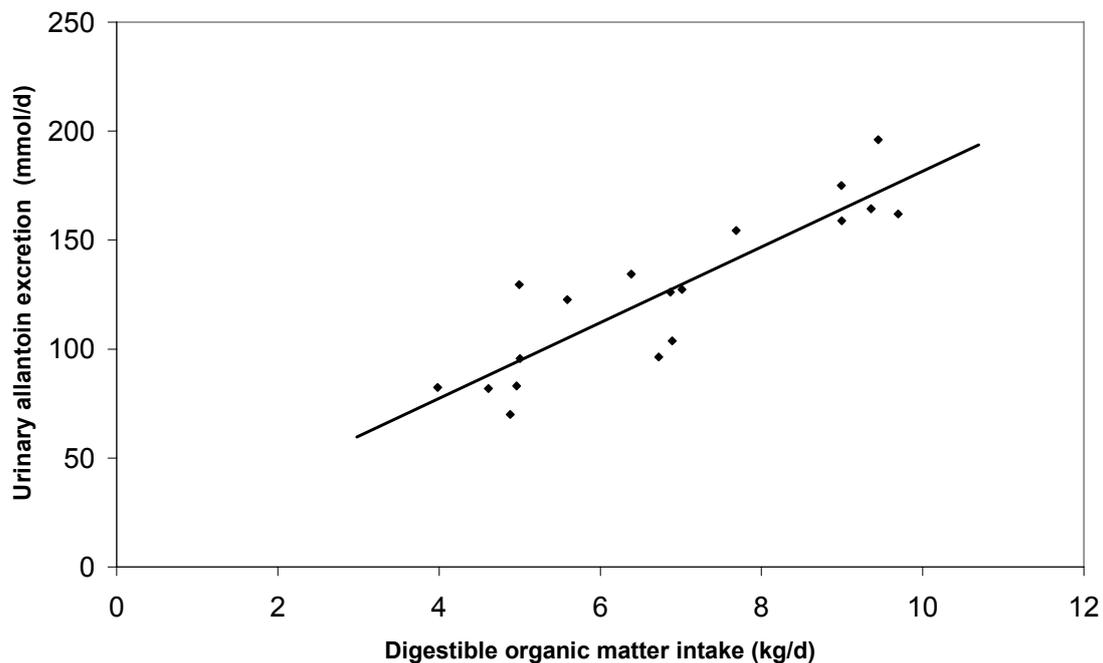
¹Standard error of least squares means

²MCP_{ALL-CØ}, MCP_{ALL-E} and MCP_{ADOMR} were estimated as outlined in the Materials and methods section

³OM truly digested in the rumen; it was assumed that digestion occurred in the rumen was 55% of OM intake (STERN et al., 1994)

A close relationship was observed between daily DOMI and urinary allantoin excretion (Figure 1) as indicated by a R^2 value of 0.785. Assuming linearity, the intercept point of the regression with the ordinate gives an estimate of the endogenous contribution to urinary allantoin excretion of 7.9 mmol/day, corresponding to $52 \mu\text{mol}/\text{kg}^{0.75}$ BW allantoin or to about $59 \mu\text{mol}/\text{kg}^{0.75}$ BW of total purine derivatives.

Figure 1: Relationship between total urinary allantoin excretion (mmol/d) and intake of digestible organic matter (kg/d) in Experiment 1 ($y = 7.94$ [SE = 15.98; $P = 0.6261$] + 17.34 [SE = 2.27; $P < 0.0001$] x ; $R^2 = 0.785$).



Experiment 2

Table 4 shows intake and digestibility of OM and urinary allantoin excretion of steers in Experiment 2. The daily OM intake ranged only from 7.4 to 9.6 kg. Advancing maturity of the ryegrass, which was paralleled by a decline of CP concentration and increasing fibre content, resulted in a marked decrease of OM digestibility and ruminal OM degradation of mixed diets. The EDOMR values of the silages also drastically declined: 73, 67, 55 and 47% for the silages harvested at 8 May, 22 May, 7 June and 19 June, respectively ($P < 0.001$; Schäfer 1996). Urinary allantoin excretion as related to DOMI declined as forage maturity at harvest advanced. The values for May silages were higher than those for June silages, and the differences became even more pronounced, when endogenous contribution was considered ($P = 0.008$; data not shown).

Table 4: Intake and digestibility of organic matter (OM) and total urinary allantoin excretion of steers fed silages of perennial ryegrass cut at four different harvest dates (Experiment 2)

	Harvest date				Effect of harvest date (P)			Contrasts (P)			SEM ¹
	8 May		7 June		19 June		May vs. June				
	22 May	9.6	8.6	7.4			May	June	June		
Intake (kg/day)											
OM	7.8	9.6	8.6	7.4							0.6
DOMI ²	6.4	7.6	6.3	5.1	0.063		0.039	0.157	0.127		0.5
ADOMIR ³	3.9	4.5	3.8	3.1	0.063		0.039	0.158	0.128		0.3
EDOMIR ⁴	5.8	6.4	4.9	3.7	0.017		0.005	0.410	0.084		0.4
TDOMIR ⁵	4.3	5.3	4.7	4.1	0.117		0.234	0.068	0.179		0.3
Digestibility (%) ⁶	82.0	79.0	74.0	69.8	0.005		0.001	0.210	0.094		1.5
Allantoin											
(mmol/day)	149	155	105	80	0.002		< 0.001	0.619	0.081		8.5
(mmol/kg DOMI)	23.5	20.6	17.2	15.7	0.043		0.002	0.226	0.518		1.6

¹Standard error of least squares means

²Digestible OM intake

³OM apparently digested in the rumen; it was assumed that 60% of whole-tract OM digestion occurred in the forestomachs (Schröder et al., 1989; Südekum et al., 1992, 1994; Schröder et al., 1997)

⁴OM effectively degraded in the rumen; derived from *in sacco* values of the silages assuming a passage rate of 5%/h (SCHÄFER, 1996)

⁵OM truly digested in the rumen; it was assumed that digestion occurring in the rumen was 55% of OM intake (STERN et al., 1994)

⁶OM apparently digested in the whole tract

The stage of forage maturity at harvest had a major influence on the amounts of MCP produced and also on the efficiencies of MCP synthesis as derived from the two approaches based on urinary allantoin excretion (Table 5).

Table 5: Amounts (g/day) and efficiencies of rumen microbial crude protein (MCP) synthesis in steers fed diets containing silages of perennial ryegrass cut at four different harvest dates estimated on the basis of urinary allantoin excretion or organic matter apparently digested in the rumen (Experiment 2)

	Harvest date				Effect (P) of harvest date	Contrasts (P)		SEM ¹
	8 May	22 May	7 June	19 June		May vs. June	June	
MCP ² (g/day)								
MCP _{ALL-CØ}	630	663	363	211	0.002	< 0.001	0.666	50.5
MCP _{ALL-E}	763	802	438	254	0.002	< 0.001	0.666	61.3
MCP _{ADOMR}	712	834	699	565	0.063	0.039	0.157	53.4
Efficiency of MCP synthesis (g N/kg TDOMR ³)								
MCP _{ALL-CØ}	24	20	13	8	0.002	< 0.001	0.199	1.7
MCP _{ALL-E}	29	24	15	10	0.002	< 0.001	0.201	2.0
MCP _{ADOMR}	26	25	24	22	0.004	0.001	0.196	0.5

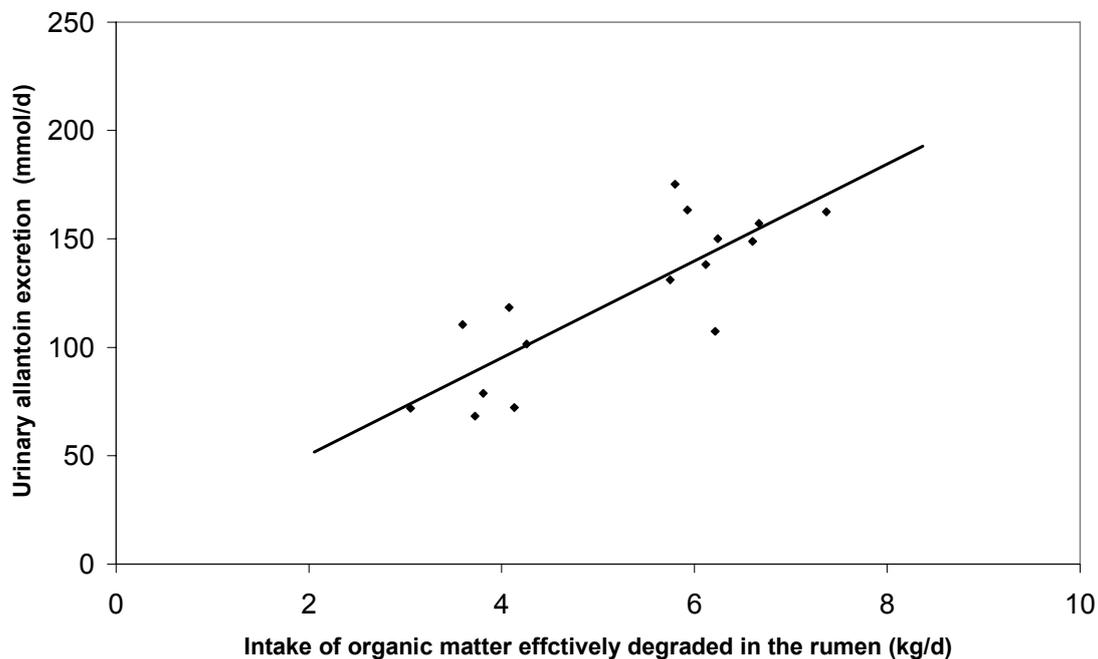
¹Standard error of least squares means

²MCP_{ALL-CØ}, MCP_{ALL-E} and MCP_{ADOMR} were estimated as outlined in the Materials and methods section

³OM truly digested in the rumen; it was assumed that digestion occurred in the rumen was 55% of OM intake (STERN et al., 1994)

The diet based on silage from the earliest harvest date (8 May) had the highest efficiency of MCP synthesis which then gradually declined by 14% (22 May), 46% (7 June) and 65% (19 June) with advancing forage maturity at harvest (Table 5). In Experiment 2, DOMI could not sufficiently explain the variation in urinary allantoin excretion. For example, values of estimated rumen MCP synthesis differed by more than 35% between diets “8 May” and “7 June” (23.5 versus 17.2 mmol allantoin/kg DOMI), although the DOMI of these two diets was almost the same. Therefore, EDOMR and not DOMI was assumed to exert a major impact on allantoin excretion. Figure 2 presents the relationship between urinary allantoin excretion and EDOMR. Generally the function shows a proper appearance as it had a satisfactory coefficient of determination ($R^2 = 0.695$). The endogenous contribution to daily urinary allantoin excretion derived from this equation is 5.9 mmoles, corresponding to 44 $\mu\text{mol}/\text{BW}^{0.75}$ allantoin or to about 50 $\mu\text{mol}/\text{BW}^{0.75}$ total purine derivatives).

Figure 2: Relationship between total urinary allantoin excretion (mmol/d) and intake of organic matter effectively degraded in the rumen (kg/d) in Experiment 2 ($y = 5.93$ [SE = 21.24; $P = 0.7841$] + 22.32 [SE = 3.95; $P < 0.0001$] x ; $R^2 = 0.695$).



4. Discussion

The objectives of this study were to evaluate effects of amount of intake alone or in combination with changes in stage of forage maturity and thus, in chemical composition of diets, on urinary allantoin excretion, which was then used to evaluate different methods of estimating MCP synthesis. The rationale for our approach was that, based on the comprehensive review by Shingfield (2000), MCP synthesis itself and estimates of MCP synthesis based on urinary excretion of purine derivatives still lack a more precise investigation of factors like diet composition and feed intake. Several researchers have shown that efficiency of MCP synthesis can be influenced by feeding level and diet composition (Isaacson et al., 1975; Clark et al., 1992; Meng et al., 1999). Energy and nutrient supplies to rumen microorganisms are of major importance because they influence (I) bacterial lysis (Meng et al., 1999) and predation of bacteria by protozoa (Clark et al., 1992) and (II) the share of nutrient consumption for maintenance (Hespell and Bryant, 1979) of rumen microbes. Besides energy and CP contents of diets additional factors have been reported to influence growth rate of rumen microbes, namely synchrony between energy and protein supply (Beever, 1993; Casper et al., 1994), energy spilling if CP supply is deficient (Van Kessel and Russell, 1996) and ruminal pH (De Veth and Kolver, 2001).

In the light of these general considerations, it appears that the non-constant efficiency of MCP synthesis as indicated by the results of the two experiments based on daily urinary allantoin excretion reflected the true situation. Although in Experiment 1 no consistent difference between treatments was observed, numerically an improvement of efficiency of MCP synthesis was observed when intake changed from the low to the medium level, whereas the increase from the medium to the high level was negligible. This observation can be explained by decreasing marginal utilization of each additional unit of energy and nutrients at increasing energy supply as the proportion of maintenance costs concurrently becomes less important (Hespell and Bryant, 1979). Unlike Experiment 1, a marked improvement of efficiency of MCP synthesis was observed in Experiment 2. Although the variation in DM intake was much less in this experiment than in Experiment 1, the chemical composition and thus nutrient amounts and ratios available for rumen microbes changed drastically with advancing forage maturity. Czerkawski (1986) and Sinclair et al. (1993) have reported that both, amount and synchrony, of nitrogen and energy supply are influencing rumen microbial growth. Using tabulated values of ruminal degradability of CP (Universität Hohenheim – Dokumentationsstelle, 1997), it can be calculated that contents of ruminally degradable CP of mixed

diets declined by 6%, 20% and 30% (22 May, 7 June and 19 June) in relation to the diet containing silage from the harvest on 8 May. As a consequence of the attempt to keep the CP contents of the diets constant, we used increasing proportions of less ruminally degradable soybean meal CP replacing rapidly and highly degradable grass silage CP in the diets containing grass silage from later harvest dates. Moreover, the decrease of CP contents of the grasses was much larger than the decrease in the intake of EDOMR. Therefore, it appears more likely that ruminally degradable CP, i.e., nitrogen supply, and not ruminally degradable OM was limiting the growth of microbes in the rumen of the steers fed the diets containing silages from later (June) harvests.

Values for estimated MCP synthesis (g/d) and the efficiency of MCP synthesis (g N/kg TDOMR) differed between the three approaches in both experiments (Tables 3 and 5). Moreover, the drop of ruminal degradability of OM with advancing forage maturity observed in Experiment 2 was more pronounced for EDOMR than for ADOMR. Hence, ADOMR would overestimate actual energy supply to rumen microbes and would thus result in an overestimation of (efficiency of) MCP synthesis. Based on this interpretation and the theoretical considerations on efficiency of MCP synthesis pointed out above, our estimate of MCP synthesis (g/day) based on the method of Czerkawski (1986) appears to be inappropriate. On the one hand, the estimate assumes a constant microbial efficiency per unit of ADOMR (184 g/kg), on the other hand the assumption may be an oversimplification of the true situation that a constant proportion of DOMI is digested in the rumen. Therefore, results from this approach are not further considered in the discussion of efficiencies of MCP synthesis. At this point we can not decide which of the two other approaches by Chen and Ørskov (2003) and Ehrenreich (1992), respectively, was more accurate, as no direct estimation of MCP synthesis using appropriate microbial markers was conducted.

Values for efficiency of MCP synthesis (g N/kg OM fermented in the rumen) were reported to range between 24 to 36 g/kg under practical circumstances (NRC, 2001). In Experiment 2 estimated values based on urinary allantoin excretion by either method, $MCP_{ALL-C\emptyset}$ or MCP_{ALL-E} , were in the range of NRC (2001) values especially for diets containing the "May" silages. Further, it should be taken into account that DMI in our experiments were much lower than those used in the NRC (2001) database. It remains unclear, however, why values observed in Experiment 1 were lower than expected as this study was not designed to investigate factors other than DMI which could have further affected the efficiency of MCP synthesis.

In this study, a linear function was used to illustrate the relationship between DOMI and urinary allantoin excretion. This appears justified in view of the distribution of the data. Theoretical considerations, however, would favour a polynomial function. The development of efficiency of MCP synthesis with increasing feed intake may be divided into three stages: (1) At very low intakes energy and nutrients are solely or nearly solely consumed for maintenance purposes of rumen microbes; (2) Once supply exceeds maintenance costs, an increasing proportion of energy and nutrients will be utilised for growth of rumen microbes, which means a high growth rate for each additional unit of energy and nutrients at this stage; (3) With further increases in energy and nutrient supply, factors other than energy and CP may successively become limiting and hence, marginal utilisation of each additional unit would decline. Assuming this, a linear function does not seem appropriate for both high and low intakes, although we observed a proper fit of the equation within the relatively narrow range of intakes in both experiments reported herein. If our regression lines are extrapolated onto the ordinate intercept point, estimates of the endogenous contribution to total urinary allantoin excretion of 7.9 mmol/d (Experiment 1) and 5.9 mmol/d (Experiment 2) are derived and both values were not different from zero (Experiment 1: $P = 0.62$; Experiment 2: $P = 0.78$). These values were much lower than those calculated according to Verbič et al. (1990), i.e., 51.5 (Experiment 1) and 45.7 mmol/d (Experiment 2) of urinary allantoin from endogenous sources. This comparison indicates that at low feed intakes, the allantoin excretion per unit of DOMI or EDOMR is much lower than estimated from our regressions equations. This conclusion, however, is in good agreement with the theoretical considerations outlined above, further supporting the assumption that allantoin excretion from microbial yield per unit of DOMI is not constant. Hence, extrapolations beyond the range of measured values should not be applied.

5. Acknowledgements

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Chapter III

Rumen-protected choline for dairy cows:
In situ evaluation of a commercial source and literature
evaluation of effects on performance and interactions between
methionine and choline metabolism

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Animal Research (in press)

Abstract – Interactions were investigated between methionine and choline metabolism due to interchangeable methyl groups. Feeding trials on dairy cows with choline administration were re-evaluated with focus on methionine balance and possible effects of supplementary choline on methionine metabolism. In addition, *in situ* ruminal stability was estimated of a commercial choline supplement. We examined six experiments that included 11 dietary treatments. The Cornell net carbohydrate and protein system was used to estimate nutrient supply and requirements as well as methionine balance; further methionine supply was assessed according to the ideal protein concept. The re-evaluation of feeding trials confirmed positive effects of supplementary abomasally available choline on milk yield and health of dairy cows. Although these effects were mainly attributed to an elevated export of triglycerides from the liver, beneficial effects may be further caused by an exoneration of methionine metabolism by sparing S-adenosylmethionine. Therefore, effects on milk yield or other response variables of additional, abomasally available choline could serve as an indicator of methyl group deficiency and thus methionine shortage. Since from theoretical considerations demand for methyl groups in dairy cows is related to stage of lactation, requirements for methionine and methyl groups should be taken as separate entities and the latter be expressed in relation to energy balance or days in milk. Our *in situ* data indicated that Reashure choline[®] was effectively protected against ruminal degradation because at least 600 g kg⁻¹ of choline were in a rumen-protected form. In conclusion, (I) supplementary choline may improve yield of dairy cows by elevating export of triglycerides from the liver and by sparing methionine as methyl donor, and (II) the demand for methyl groups still lacks adequate consideration in design of diets for dairy cows.

Résumé – Apport de choline protégée aux vaches laitières: évaluation *in situ* d'une source commerciale, et évaluation bibliographique de ses effets sur les performances et sur le métabolisme de la méthionine.

Des essais dans lesquels de la choline avait été administrée à des vaches laitières ont été réévalués en portant une attention particulière au bilan de la méthionine et aux effets de la choline apportée en supplément sur le métabolisme de la méthionine. De plus, la stabilité dans le rumen d'un supplément commercial de choline a été évaluée *in situ*. Six essais incluant 11 traitements alimentaires ont été étudiés. Le système d'alimentation énergétique et azoté de Cornell a été utilisé pour estimer l'apport et le besoin de nutriments, et le bilan de la méthionine. En outre, l'apport de méthionine a été évalué selon le concept de la protéine idéale. La réévaluation des essais d'alimentation a confirmé l'effet positif de l'apport de choline disponible dans la caillette sur la quantité de lait produite et la santé des vaches laitières. Bien que ces effets aient été principalement attribués à une sécrétion accrue de triglycérides par le foie, des effets bénéfiques peuvent aussi être provoqués par une épargne de S- adénosylméthionine, donneuse de groupements méthyle. Les effets de la choline supplémentaire disponible dans la caillette ont pu servir comme indicateurs d'insuffisance de groupements méthyle et, ainsi, d'apport de méthionine insuffisant. Puisque, à partir de considérations théoriques, le besoin en groupements méthyle chez des vaches laitières est lié au stade de lactation, les besoins en méthionine et en groupements méthyle devraient être considérés comme des entités séparées, et ces derniers devraient être exprimés par rapport au bilan énergétique ou au stade de lactation. Nos données *in situ* ont montré qu'au moins 60% de la choline Reashure[®] apportée était effectivement protégée contre la dégradation dans le rumen. En conclusion: (i) l'apport de choline supplémentaire peut améliorer la production des vaches laitières en accroissant la sécrétion de triglycérides par le foie et en épargnant la méthionine en tant que donneuse de groupements méthyle ; (ii) le besoin de groupements méthyle n'est toujours pas suffisamment pris en compte dans le rationnement des vaches laitières.

1. Introduction

Several metabolic disorders in dairy cows occur especially during the transition period when cows may undergo a severe negative energy balance. With the onset of lactation, feed intake is increasing slowly, whereas nutrient and energy requirements are rapidly increasing. Adipose tissue is mobilised and the plasma levels of non-esterified fatty acids (NEFA) are elevated. The NEFA are utilised by several tissues but are transported to the liver as well. If uptake into the liver exceeds conversion (e.g., oxidation or synthesis of ketone bodies) and export (in the form of triglycerides (TG) via very-low-density lipoproteins (VLDL)), excess lipids are stored in the liver as TG (Bobe et al., 2004). Since several metabolic disturbances and diseases are associated with a severe negative energy balance, an optimal feeding strategy and diet composition during the transition period appear to be the best prevention of health disturbances and associated decline of milk yield. Additional approaches frequently include feed additives such as glucogenic precursors, fatty acids and others (see Overton and Waldron, 2004).

Recent research indicated that one of these feed additives, rumen-protected choline (RPC), might increase VLDL synthesis in the liver. Choline is a constituent of phosphatidylcholine (trivial name: lecithin), which is inevitably involved in VLDL synthesis (Overton and Waldron, 2004). Contrary to that in non-ruminant animals, virtually no lecithin is synthesised from feed-based choline in ruminants: choline from feedstuffs is degraded extensively in the rumen (Atkins et al., 1988; Sharma and Erdmann, 1988 and 1989). Lecithin in ruminants is usually synthesised by threefold transfer of methyl groups from S-adenosylmethionine (AdoMet) onto phosphatidylethanolamine (Pinotti et al., 2002; Snoswell and Xue, 1987). Hence, RPC may serve to spare cellular methionine (Met) as methyl donor as it delivers choline for the synthesis of lecithin.

The aims of the present study were to review current knowledge on interrelationships between Met and choline metabolism (i.e. synthesis and degradation of both, choline and methionine) and to re-evaluate Met supply of diets used in published feeding trials with supplementary choline. As only few experiments have been conducted, a detailed statistical evaluation was not possible; data were sufficient only to evaluate Met supply of diets for dairy cows in general. Additionally, an *in situ* trial was conducted to assess the effectiveness of choline protection against ruminal degradation in a commercial RPC source.

2. Material and Methods

Re-evaluation of feeding trials with supplementary choline

Input data

Diets (n = 11 treatment means) from feeding experiments (n = 6) were evaluated for balance of metabolisable energy (ME), metabolisable protein (MP) and absorbed Met and for absorbed Met as a proportion of MP. Only Holstein cows were used in the analysed experiments (Table I); cows were mostly multiparous (as far as reported). Stage of lactation at the beginning of the experiments was very heterogeneous and ranged from 28 d prior to calving to about 150 d in milk. Choline was supplemented as RPC or infused postruminally into the abomasum or duodenum. We did not consider further a short-term experiment by Grummer et al. (1987) and experiments in which choline was fed as unprotected choline chloride (Atkins et al., 1988; Erdman et al., 1984; Sharma and Erdman, 1988).

Table 1: Production and treatment data of six experiments with 11 dietary treatment means.

Route of choline administration	Days relative to calving at the beginning	Choline supply (g d⁻¹)¹	F:C²	Milk yield (kg d⁻¹)³	Source
Infusion	89 - 125	30	40:60	28.3	Sharma and Erdman (1988)*
Infusion	150	50	30:70	21.5	Sharma and Erdman (1989)
	83	30 / 60 / 90	40:60	26.7	
	64	40	40:60	31.8	
Rumen-protected	29	15 / 30 / 45	40:60	36.7	
	151	15 / 30 / 45	40:60	31.7	Erdman and Sharma (1991)
	151	15 / 30 / 45	40:60	31.8	
Rumen-protected	-28	6 / 12	54:46	38.6	Hartwell et al. (2000)
	-28	6 / 12	54:46	37.2	
Rumen-protected	-14	20	61:39	28.6	Pinotti et al. (2003)
<u>Rumen-protected</u>	<u>-21</u>	<u>11 / 15 / 19</u>	<u>54:46</u>	<u>40.0</u>	<u>Piepenbrink and Overton (2003)</u>

¹Treatments included a control group except the trial marked with * - here either choline or methionine were infused with or without

2-amino-2-methyl-1-propanol.

²Forage:concentrate ratio.

³Milk yield is reported for control groups receiving no choline except the trial marked with * - here yield of the group receiving a choline infusion without 2-amino-2-methyl-1-propanol is reported.

Concerning the chemical composition of diets and animal performance data, average milk yield was 32.3 kg d⁻¹ (standard deviation (SD) 5.7) with average concentrations of milk protein and fat of 32 g kg⁻¹ (SD 2) and 36 g kg⁻¹ (SD 5). Hence, 4% fat-corrected milk (FCM) yield was 29.5 kg d⁻¹ (SD 8.1). Dry matter (DM) intake (DMI) averaged 20.4 kg per day (SD 2.5), and diets were based upon maize products: proportion of maize, fed as forage plus concentrate, ranged from 490 to 900 g kg⁻¹ of DMI. Maize silage either constituted the sole forage (7 treatments) or was fed as a mixture with others (mostly lucerne as silage or hay). Concentrates accounted for 380 to 700 g kg⁻¹ DMI and mainly consisted of maize grain, with soybean meal or maize gluten feed as major protein supplements. The energy density (net energy for lactation) of the diets averaged 7.3 MJ kg⁻¹ DM (SD 0.3) and the mean crude protein concentration was 157 g kg⁻¹ DM (SD 18) with an average of 387 g kg⁻¹ crude protein (SD 37) as ruminally undegraded protein.

Calculations

Metabolisable energy, MP and Met balances were calculated using the Cornell net carbohydrate and protein system (CNCPS, Fox et al., 2004). Data of ME, MP and Met balances are reported as the ratio of excess or shortage of supply to requirements. Feeds listed in the publications were matched with feeds from feeding model libraries. Where available, crude nutrient concentrations of feedstuffs were modified to reflect actual, published dietary concentrations. Although the output of the CNCPS may not reflect the actual data for each diet, it provides reasonable overall estimates.

Supply of Met was assessed using two concepts. First, digestible Met at 25 g kg⁻¹ of MP as recommended by the ideal protein concept (Doepel et al., 2004; Rulquin et al., 1993) was used. In the output of the CNCPS, this number is reported as percent absorbed Met of MP. Second, the Met balance computed by the CNCPS was used as a comparative value.

In situ trial

Animals and diet

Three six-year-old German Red Pied steers were used, weighing 940 kg on average. Each steer was fitted with a 10 cm internal diameter ruminal cannula (Model 1C, Bar Diamond, Parma, ID, USA) and housed in individual tie stalls on rubber mattresses with free access to water. Steers were offered a diet consisting of grass silage, wheat straw, and mixed concentrates in the ratio (g kg⁻¹ DM) of 560:140:300 in two equal portions at 0700 and 1900 hours. The DMI corresponded to approximately 110% of the estimated ME requirements for maintenance (AFRC, 1991).

In situ procedure

Ruminal DM and choline disappearance of a commercial RPC source¹ was determined using an *in situ* procedure described in detail elsewhere (Shannak et al., 2000). Briefly, polyester bags (5 cm x 10 cm, R510, Ankom Technology, Macedon, NY, USA) with a pore size of 50 μm were used. Duplicate samples of RPC were incubated in the rumens of the three steers, and about 1.3 g of fresh matter were placed in each bag. Until the start of incubation, bags were stored frozen (-18 °C).

Before incubation, each bag was sealed with a commercial cable binder (10 cm length); then, bags were clamped to a cylindrical anchor weight (800 g), which was tied to an 80 cm long main line outside the fistula. Prior to incubation, the bags were soaked in warm water (40 °C) for 10 minutes. All bags were inserted into the ventral sac of the rumen at 0700 hours directly before feeding. Incubation periods were 2, 4, 6, 8, 16, 24, 48, 72 and 336 hours. After ruminal incubation, bags were immediately immersed in ice water to minimise microbial activity. Bags were then washed with cold water in a washing machine for 20 minutes in order to remove feed particles adhering to the bag surface. Water was exchanged five times during washing, and no spin cycle was used.

Zero time disappearance values (0 hours) were obtained by washing pre-soaked, unincubated bags in a similar fashion. Bags were freeze-dried before chemical analysis.

Laboratory analysis

The DM of feeds consumed by the steers and that of RPC and freeze-dried residues after ruminal incubation were determined by oven-drying at 105 °C overnight. The RPC and the residues after ruminal incubation were analysed for choline contents according to the method of Takayama et al. (1977). Choline was oxidised to betaine by choline oxidase with the simultaneous production of hydrogen peroxide, which by oxidation couples 4-aminoantipyrine and phenol to yield a chromotogen with a maximum absorbance at $\lambda = 500 \text{ nm}$. Choline chloride (approx. 99%; C7017, Sigma-Aldrich Chemie GmbH, Munich, Germany) was used as a standard. For RPC, the method of Takayama et al. (1977) had to be modified to overcome the fatty acid encapsulation such that the sample was heated to about 60 °C before further processing.

¹Reashure choline[®]; Balchem Inc., Slate Hill, NY, USA.

3. Results

Re-evaluation of feeding trials with supplementary choline

Data of MP and ME balance as well as Met supply are reported in Table II. In only two of six trials (three dietary treatments) energy supply was lower than requirements, MP was deficient in three trials (four dietary treatments), and the Met balance calculated by the CNCPS was negative in two trials (three dietary treatments). Contrary, the percentage of Met was less than 21 g kg⁻¹ of MP for all dietary treatments and thus below the threshold formulated by the ideal protein concept.

Effects of choline supplementation on performance variables varied. For 10 treatments significant effects were observed, mostly increased milk yield (n = 6), sometimes accompanied by increased contents or yields of protein (n = 3 and 1) or fat (n = 3 and 3); decreased yields were observed in three cases. For the remaining one treatment, performance only tended to be higher than the control.

Table 2: Balance (expressed as percent over or below requirements) of metabolisable energy (ME), metabolisable protein (MP) and absorbed methionine (Met) and observed effects of supplementary choline (fed in rumen-protected form or infused posttruminally) relative to control in 6 experiments

Source	Nutrient supply				Observed effects of supplementation
	Balance (%)			Met (% of MP)	
	ME	MP	Met		
Sharma and Erdman (1988)*	+ 20.5	+ 2.5	+ 15.4	2.01	M+, P%+, Pkg+, F%+; Fkg+, FCM+ P%+, F%+, Fkg+
Sharma and Erdman (1989)	+ 22.3	+ 5.3	+ 17.9	2.00	M+
Erdman and Sharma (1991)	+ 13.7	- 13.1	- 4.7	1.99	Trends only
Hartwell et al. (2000)	+ 22.7	+ 0.9	+ 4.4	1.85	M+
Pinotti et al. (2003)	+ 15.6	+ 2.7	+ 11.1	1.95	FCM+, P%-, F%-
Piepenbrink and Overton (2003)	- 25.6	- 16.6	- 8.9	1.98	M+, P%- M-, P%+

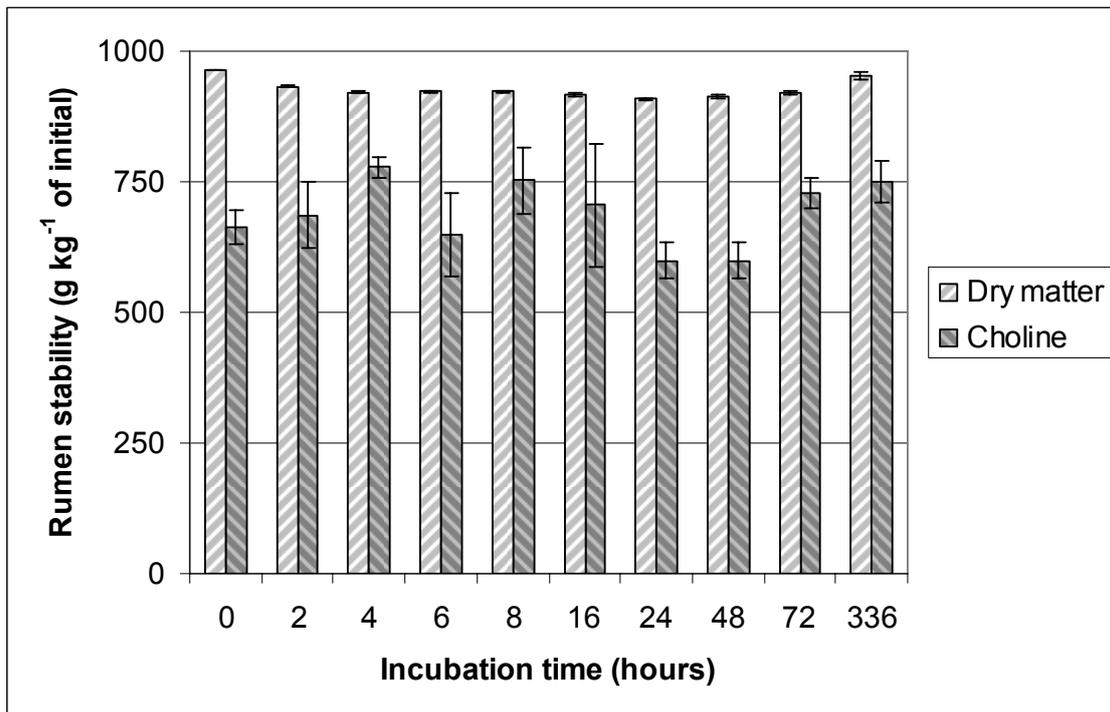
NS = not significant; +/- = significant [$\alpha = 0.05$] change as compared with control diet in milk yield [M], protein yield [Pkg], protein percentage [P%], fat yield [Fkg], fat percentage [F%] and 4% fat-corrected milk yield [FCM].

*Upper line: with 2-amino-2-methyl-1-propanol application, lower line without 2-amino-2-methyl-1-propanol application.

In situ trial

The average choline content of RPC was 234 g kg^{-1} . This value corresponded well with that given by the manufacturer (250 g kg^{-1}). Ruminal DM and choline stability of RPC are reported in Figure I.

Figure I: Ruminal dry matter and choline stability of a commercial source of rumen-protected choline (RPC, based on 6 observations [3 steers with two replicates per steer] per incubation time). Vertical bars indicate the standard error.



The DM losses remained below 100 g kg^{-1} at all incubation times: Washout losses (0-hour disappearance value) were about 36 g kg^{-1} and additional losses occurred at the beginning of the incubation. At longer periods of incubation total DM disappearance values decreased. Regarding choline stability of RPC, a rapid initial disappearance (0 h) of choline occurred, after which no considerable further disappearance was observed. Overall, choline disappearance values never exceeded 400 g kg^{-1} of the amount incubated irrespective of duration of incubation.

4. Discussion

Re-evaluation of feeding trials with supplementary choline

The beginning of lactation is one of the most crucial periods in the lactation cycle of dairy cows. As some excellent reviews have been recently published concerning the role of lecithin (Pinotti et al., 2002) and liver metabolism of dairy cows during this time (Bobe et al., 2004; Overton and Waldron, 2004) only a brief account will be given here.

At the beginning of the lactation cycle the plasma level of NEFA originating from mobilisation of adipose tissue is elevated - mainly due to a negative energy balance (see Overton and Waldron, 2004). If uptake of NEFA into the liver exceeds liver utilisation (oxidation and synthesis of ketone bodies) and export of fatty acids from the liver in the form of VLDL, excess TG are stored in hepatocytes (see Bobe et al., 2004). An accumulation of TG (i.e., fatty liver, hepatic lipidosis) may lead to an impaired capacity of the liver for gluconeogenesis (Cadórniga-Valiño et al., 1997; Strang et al., 1998) and would result in reduced performance. In general, hepatic lipidosis is associated with decreased health status (see Bobe et al., 2004). Hence, efforts should focus on minimising storage of TG in the liver. An optimal nutrition serves to decrease the magnitude of the negative energy balance but cannot solve the problem of excess uptake of TG into the liver in high-yielding dairy cows. In general, VLDL secretion of hepatocytes of ruminants is much lower than that of non-ruminants, and the secretion rate appears to be much lower than the rate of hepatic TG synthesis (see Grummer, 1993). Therefore, it is advisable to avoid any limitation of VLDL synthesis. The rate limiting steps of VLDL synthesis are not entirely clear yet; especially synthesis of lecithin and apolipoproteins may be limiting (Bernabucci et al., 2004; see Overton and Waldron, 2004). While the influence of the availability of apolipoproteins is largely speculative to date, the influence of lecithin, first shown by Yao and Vance (1988) in trials with rats, was confirmed in several experiments with dairy cows (see Overton and Waldron, 2004; see Pinotti et al., 2002). Since dietary choline is degraded virtually completely in the rumen, lecithin may be quantitatively synthesised endogenously in ruminants by threefold transfer of methyl groups from AdoMet onto phosphatidylethanolamine.

To date most of the potential application of choline in the nutrition of transition dairy cows has focused on its role in lipid metabolism (Overton and Waldron, 2004). Pinotti et al. (2002) presumed that the extra demand during lactation for methyl groups for synthesis of lecithin and other compounds may be met by enhanced de novo synthesis of methyl groups via the tetrahydrofolate system. A recent trial validated the hypothesis Pinotti et al. (2004), however, the quantitative importance of this pathway still needs to be investigated. Emmanuel and

Kennelly (1984) on the other hand reported that in lactating goats more than one fourth of total Met (280 g kg^{-1}) was utilised for choline synthesis. Hence, rate of lecithin synthesis may be limited due to a deficient supply of Met as assumed by some workers who reported that supplementary Met affected the hepatic VLDL balance (Durand et al., 1992; Pisulewski et al., 1996). Thereby, milk yield of dairy cows may decline: Export of TG is depressed by insufficient supply of methyl groups from AdoMet for the synthesis of lecithin, and rate of gluconeogenesis is lowered due to an accumulation of TG in liver hepatocytes. Since as much as half of Met requirements may be met through remethylation of homocysteine (Donkin, 2002), it seems reasonable to assume that a deficient methyl group supply from AdoMet indicates also a shortage of Met.

Positive effects of supplementary choline were observed with 10 of 11 dietary treatments that were re-evaluated in this study. Based on the ideal protein approach, all diets were deficient in Met supply ($< 25 \text{ g kg}^{-1}$ of MP), whereas the Met balance calculated by the CNCPS was negative for only three dietary treatments. It seems probable that the concept of the ideal protein reflects methyl group supply from AdoMet more appropriately than the Met balance does. The amino acid sub-model of the CNCPS uses a factorial approach for determining the daily requirements for and supply of absorbed amino acids (O'Connor et al., 1993), whereas Met requirements in the ideal protein concept were derived from dose-response trials with predominantly early-lactation cows. Since an elevated demand for methyl groups likely occurs in the transition period when the plasma level of NEFA increases, the latter approach appears more adequate. Additionally, in a comparison of systems for the protein quality of diets for dairy cows (Piepenbrink et al., 1998), the ideal protein concept most accurately predicted milk protein percentages and production, and was superior to other models such as the CNCPS.

In three of the 10 dietary treatments with significant effects of choline supplementation on animal performance, elevated protein contents or yields were also observed. Those effects, likely due to elevated casein contents, were frequently observed in trials with supplementary abomasally available Met and lysine (see Schwab, 1996). Hence, at least in case of these treatments, supplementary choline presumably exonerated Met metabolism, leading to elevated protein contents or yields. Several mechanisms may be involved with such a response. First, RPC may serve to spare methyl groups and hence AdoMet as methyl group donor usually necessary for the synthesis of lecithin. Second, RPC may serve as methyl group donor for remethylation of homocysteine via its metabolite betaine (see Pinotti et al., 2002; Puchala et al., 1994). Third, betaine may also spare Met by substituting for AdoMet as methyl group donor in some metabolic processes (Puchala et al., 1996). The latter two mechanisms are still hypothetical. Trials investigating this objective in general are rare, and to our knowledge no study on dairy cows has been published so far.

Based on the concept of the ideal protein, all diets re-evaluated in this study were deficient in Met supply, and that is in line with earlier experiments that showed that Met can become first limiting in maize silage-based diets (Pisulewski et al., 1996). Thus - and from theoretical considerations regarding the transition period - it seems reasonable to assume that a shortage of supply of methyl groups from AdoMet is common in transition dairy cows – at least, if diets are based on maize. Therefore, the question arises as to how the demand for methyl groups can best be met – either supplying extra RPC or extra Met?

Met plays a crucial role in mammalian metabolism: as an essential amino acid, as a precursor of cysteine biosynthesis and as the key intermediate in methyl group transfer (Lobley et al., 1996). Thus, in case of Met shortage, rumen-protected Met seems to be a more appropriate supplement, as RPC may only serve to spare methyl groups. Moreover, a direct excretion of Met metabolism by RPC in dairy cows has not been clearly shown to date. On the other hand, supplementary RPC appears to be the better choice, if an elevated lecithin synthesis and elevated VLDL synthesis in hepatocytes during the transition period is the primary goal. It appears reasonable to conclude that demand for methyl groups for synthesis of lecithin will vary depending on the metabolic and energetic status of the dairy cow. Hence, it seems useful to separate demand for methyl groups from Met requirements and to express demand for methyl groups in relation to the actual energy balance. As energy balance of dairy cows especially depends on the stage of lactation, demand for methyl groups could be expressed related to days in milk as an approximation of overall energy balance.

To verify this assumption, trials should be conducted with cows classified by energy balance and (or) days in milk, and diets should be based on maize. As demand for methyl groups is presumably best met by supplementary choline, RPC should be used in dose-response experiments. Milk yield and Met supply should be considered as other factors of influence. Further, possible interactions with methyl groups delivered by *de novo* synthesis via the tetrahydrofolate system should not be ruled out.

In situ trial

Ruminal dry matter losses of RPC were low, relatively constant, and never exceeded 100 g kg⁻¹. These numbers are in good agreement with results of an *in vitro* trial by Kung et al. (2003). The decline of losses at incubation times longer than 24 hours is somewhat surprising and might be related to microbial infiltration of bags. Ruminal choline stability showed fluctuations that might be related to variations in analysis of choline contents of RPC because of a possibly uneven distribution of choline within samples of RPC. Despite the greater variance of choline contents of RPC, it can be stated that ruminal choline disappearance never exceeded 400 g kg⁻¹ and most of this disappearance occurred as washout loss; no

trend towards progressing disappearance at longer incubation periods was observed. This again confirms the *in vitro* data of Kung et al. (2003).

5. Conclusion

Supplementary RPC showed positive effects on health and/or yield in several feeding trials on dairy cows which is mainly attributed to an elevated synthesis of VLDL in the liver. Data possibly indicate that supplementary RPC exonerates Met metabolism due to interchangeable methyl groups. The extent of beneficial effects of supplementary RPC presumably depends on the energy balance of the dairy cow. Vice versa, supplementary rumen-protected Met may have positive impacts on choline metabolism. Both assumptions, however, remain hypothetical to date because no experiments on dairy cows have been published investigating this mutual influence. Because beneficial effects of supplementary RPC, mainly attributed to the supply of additional methyl groups, have been frequently observed, we conclude that the demand for methyl groups in dairy cows to date lacks adequate consideration. If a deficiency of methyl groups in dairy cows is likely to occur, RPC appears to be a good choice for supplementation; its encapsulation technique prevented around 600 g kg⁻¹ of its choline from ruminal degradation. Since this protection lingers at slow dilution rates, it is applicable as well in the transition period, when low DMI are observed frequently.

In conclusion, further nutrition experiments are required for a better understanding of the interrelationship between Met and choline metabolism and for a quantification of the demand for methyl groups of dairy cows especially during the transition period.

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Chapter IV

Predicting the metabolisable energy concentration
of whole-crop silages for ruminants

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(Submitted for publication)

Abstract – This study was undertaken to improve the prediction of the metabolisable energy (ME) contents of whole-crop silages made from small grain cereals or grain legume because the currently used equation ($ME = 11.57 - 0.00977 \times \text{crude fibre} - 0.00711 \times \text{ash} + 0.00621 \times \text{crude protein}$; ME given as MJ/kg dry matter (DM) and proximate constituents expressed as g/kg DM), which was based on 114 digestibility trials, has a relatively low coefficient of determination ($r^2 = 0.55$). Data of a total of 70 diet means from standard digestibility trials conducted in Germany were utilised (I) to validate the currently used prediction and (II) to evaluate possibilities to enhance the accuracy of the prediction utilising several multiple linear models. Proximate constituents and DM were utilised as general independent variables, and starch and organic matter solubility in cellulase as candidate additional independent variables. Geographic region of crop growing and type of grain used for silage production were added as candidate classification factors. Utilisation of contents of proximate constituents and DM resulted in a poor prediction of the energy density ($r^2 = 0.26$, standard error of estimate = 0.39). Starch content as an additional independent variable did not yield a significant enhancement, whereas organic matter solubility in cellulase appeared promising. Insertion of geographic crop growing region and type of grain yielded significant improvements of the accuracy of prediction – the latter presumably mainly due to differences between pea and cereal silages. Therefore, separate equations would be desirable taking into account type of grain and geographic region of crop growing to obtain better predictions than currently available for ME contents of whole-crop silage based on concentrations of proximate silage constituents. Since this may not be feasible for advisory services due to high additional efforts or simply due to the lack of accessible information, improvements may be achieved by means of specific equations for definite geographic regions. Moreover, other analytical procedures than proximate constituents may produce improved predictions for the energy density of whole-crop silages. Validation of the current equation with our dataset showed an average underestimation of circa 0.5 MJ ME/kg DM compared with ME concentrations derived from digestibility trials on sheep. Although not satisfying, this prediction should be used further on because our dataset was not sufficient to establish an improved prediction. Future research should aim at better understanding possible factors influencing the energy density of whole-crop pea and small grain cereal silages.

Abbreviations: ADF, Acid detergent fibre; ADL, Acid detergent lignin; aNDF, Neutral detergent fibre (using heat stable amylase and with residual ash); DM, Dry matter; ELOS, Organic matter solubility in cellulase; ME, Metabolisable energy; NEL, Net energy for lactation; GR, Geographic region of growing; TOG, Type of Grain; WCS, Whole-crop silage

1. Introduction

Whole-crop silages (WCS) from small grain cereals and grain legumes have found considerable interest in ruminant feeding as an alternative to maize silage particularly in cooler regions with less favourable growing conditions for maize or when intensive maize cropping has caused ecological problems such as increased nitrate loss or reduced ground cover (Greef and Taube, 1998). To provide metabolisable energy (ME) values that are needed for the formulation of diets containing WCS, advisory services throughout Germany use laboratory-based techniques to estimate the ME from proximate constituents from the following equation (Südekum and Arndt, 1998): Metabolisable energy (ME; MJ/kg dry matter (DM)) = $11.57 - 0.00977 \times \text{crude fibre} - 0.00711 \times \text{ash} + 0.00621 \times \text{crude protein}$ (proximate constituents expressed as g/kg DM). This procedure allows simple and rapid predictions of the energy density of WCS. Only, the current prediction lacks accuracy since its coefficient of determination values only 55% and the residual variance (relative standard deviation) is 5.6%. These values are too high if WCS are to be fed to dairy cows, when exact information is necessary. Efforts must be undertaken to improve the accuracy of prediction.

The objectives of this study were (I) to validate the actual prediction equation and (II) to evaluate possibilities to enhance the accuracy of the prediction by use of additional independent variables and classification factors. Since the dataset originally used to establish the actual prediction (Südekum and Arndt, 1998) was not available any more, newer data from standard digestibility trials on sheep fed on WCS were used.

2. Material and methods

Input data

Data were available of diets (n = 70 treatment means; 48 previously unpublished) from standard digestibility trials on wethers using four types of grain (TOG) which were grown in three different geographic regions (GR) in Germany (see Table 1).

Table 1: Frequencies of 70 whole-crop silages cross tabulated by type of grain and geographic region of growing

Type of grain	Geographic region				Total number
	East	North	South		
Barley	3 ^a	5 ^{c,f}	5 ^{d,g,i}		13
Peas	11 ^a	1 ^c	0		12
Wheat	1 ^a	19 ^{b,c,e}	5 ^{d,g,i}		25
Triticale	17 ^a	1 ^c	2 ^h		20
Total	32	26	12		70

^a F. Hertwig (unpublished).

^b J. Thaysen (unpublished).

^c University of Kiel (K.-H. Südekum, unpublished).

^d Kirchgeßner et al. (1989).

^e Mannerkorpi and Brandt (1993).

^f Mannerkorpi and Brandt (1995).

^g Schwarz et al. (1989).

^h Spann et al. (2002).

ⁱ Staudacher et al. (1982).

Concentrations of DM, ash, organic matter, crude fibre, crude lipid, crude protein, ME and net energy for lactation (NEL; both, ME and NEL calculated in accordance to Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie, 1991) were available for all silages. In data subsets, contents of starch (n = 55) and detergent fibre fractions (n = 19), and organic matter solubility in cellulase (ELOS; n = 39) were also known. Contents of starch was not available in 11 pea-based and one triticale WCS from eastern Germany, two silages from southern Germany (wheat and barley) and one pea WCS from northern Germany. The contents of ELOS was not available for 17 silages from northern Germany (13 wheat, 3 barley

and 1 pea WCS), all 12 silages from southern Germany and 2 pea-based WCS from eastern Germany. Data on detergent fibre fractions were only available for 19 WCS (13 wheat, 5 barley and 1 pea silage) from northern Germany. Data for spring barley and winter barley were pooled; wheat silages were solely made from winter wheat. Chemical composition of the silages is reported in Table 2.

Table 2: Chemical composition and energy values for ruminants of 70 whole-crop silages
(g/kg of dry matter unless otherwise indicated)

Proximate constituent	unit	Mean (SD)		Range	
Dry matter (DM)	g/kg	386	(95)	196	- 687
Ash		59	(19)	37	- 113
Crude fibre		248	(32)	156	- 307
Crude lipid		20	(4)	10	- 31
Crude protein		101	(21)	71	- 179
Nitrogen free extracts		578	(50)	446	- 681
Organic matter (OM)		899	(21)	821	- 929
Starch ^a		181	(73)	12	- 398
OM digestibility coefficient		0.697	(0.035)	0.599	- 0.769
ELOS ^b		624	(41)	531	- 710
Metabolisable Energy	MJ/kg DM	9.9	(0.5)	8.7	- 11.0
Net energy for lactation	MJ/kg DM	5.9	(0.3)	5.0	- 6.7

SD = Standard deviation.

^a Data on starch contents was available for 56 of 70 silages.

^b OM solubility in cellulase; data was available for 39 of 70 silages.

Animals, diets and feeding

In general, all standard digestibility trials unpublished up to now were conducted in accordance with the recommendations specified by the German Society for Nutrition Physiology

(Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie, 1991) and the ME values of the WCS were calculated from the digestibility coefficients of the proximate constituents according to Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie (2001). Mature wether sheep, at least four per diet, were fed at approximately 110% of the estimated ME requirements for maintenance (Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie, 1995). Feed-grade urea (Yara Brunsbüttel GmbH, Brunsbüttel, Germany) as N supplement was mixed into the WCS before each feeding to maintain 13% crude protein in the DM of all WCS diets without supplying extra energy. A mineral-vitamin supplement was given to the sheep to cover daily requirements. Water was freely available at all times. After a preliminary adaptation period of 14 days, faeces were collected quantitatively over a period of 10 days. To minimise losses of volatiles, total faeces were collected twice daily around feeding times and stored frozen (-18°C) until being mixed and homogenised at the end of the sampling period. Feed refusals, if any, were collected twice daily. Feed samples and feed refusals were frozen immediately after collection (-18°C).

The crops for making WCS from northern Germany (previously unpublished) were grown, harvested and ensiled by local farmers. All silages were directly cut by precision chop-forage harvesters. Silages in quantities of approximately 120 kg DM were collected after opening the silos and used for the digestibility trials.

Crops for East-German WCS were grown at an experimental farm near Paulinenaue and also directly cut by precision chop-forage harvesters. The whole crops were ensiled and silos opened before the start of the digestibility trials.

Laboratory analyses

The DM of silages, feed refusals and faeces were determined by freeze-drying and subsequent oven-drying at 105 °C overnight. For all analyses silages, feed refusals and faeces were either freeze-dried or oven-dried and successively ground in mills with 3 and 1 mm screens and, for starch analysis, with a 0.2 mm screen. All feedstuffs, feed refusals and faeces were analysed for ash, crude fibre, crude lipid and crude protein. In subsets of all silages available for further analyses, contents of starch, ELOS and detergent fibre fractions were also determined. Ash was determined by ashing in a muffle furnace at 550 °C overnight (16 h). The following analytical procedures were used according to "Verband Deutscher Land-

wirtschaftlicher Untersuchungs- und Forschungsanstalten“ (VDLUFA; Bassler, 1988, 1993). The N was determined using a standard Kjeldahl procedure using Cu^{2+} as a catalyst (method 4.1.1) and crude protein calculated as $\text{N} \times 6.25$, crude fibre as the organic residue after treatment with diluted H_2SO_4 and KOH solutions (method 6.1.1), crude lipid as petroleum ether extract by the Soxhlet method (method 5.1.1), and ELOS (method 6.6.1) was determined using a two-step pepsin/ HCl and cellulase ("Onuzuka R-10" from the yeast *Trichoderma reesei*) procedure. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) analyses were conducted according to Van Soest et al. (1991). Detergent fibre analyses were performed without the use of decalin. Sodium sulfite was omitted and triethylene glycol was used instead of 2-ethoxyethanol in the NDF procedure and NDF on WCS and feed refusals was determined with α -amylase. The NDF and ADF values are expressed with residual ash and therefore hereafter designated aNDF and ADF. Starch content was determined by enzymatic hydrolysis of starch to glucose, employing a heat-stable α -amylase (Termamyl 120 L; Novo Industrials, Bagsværd, Denmark) as a starch-solubilising agent (Brandt et al., 1987).

Calculations and statistical analysis

The DM contents of silages were corrected for losses of volatiles during drying (Weißbach and Kuhla, 1995). The DM of silages from eastern Germany was corrected by the equation that utilises chemical analyses of silages and their residuals after drying. Silages from northern Germany were corrected by the simple equation that does not demand any chemical analyses beyond proximate constituents (Weißbach and Kuhla, 1995: $\text{DM}_{\text{corrected}} = 20.08 + \text{DM}_{\text{original}} \times 0.975$; all values g/kg DM). This equation was also utilised to correct WCS from southern Germany for losses of volatiles. However, since here no other information than that reported in the publications was available, contents of digestible organic matter and energy density had to be corrected by approximation: organic matter and NEL were corrected following the correction values for similar silages given by Weißbach and Kuhla (1995). The ME content was then adjusted by the values derived from the WCS from northern German by applying correction values in relation to DM contents.

As a first step, data of feeding trials evaluated in this study were used to validate the accuracy of the prediction equation for ME of WCS (Südekum and Arndt, 1998):

$\text{ME (MJ/kg DM)} = 11.57 - 0.00977 \times \text{crude fibre} - 0.00711 \times \text{crude ash} + 0.00621 \times \text{crude protein}$

(Proximate constituents expressed as g/kg DM) $r^2 = 0.55$

Secondly, silages were analysed for factors influencing the energy density: Multiple linear regressions were conducted using ME as response variable and proximate constituents (ash, crude fibre, crude lipid, and crude protein) and DM as basic independent variables (denoted as β). In the first approach only contents of proximate constituents and DM were used as independent variables. Additional approaches were conducted to examine potential benefits of starch and/or ELOS as additional independent variables and TOG and/or GR as additional classification factors. The aNDF contents or other detergent fibre fractions of WCS were not included in our evaluation because this data was only available for a limited number of silage materials and because it was shown for maize silages that variations in cell wall components were not closely related to differences of in vivo digestibility (Givens et al., 1995). Presumably, this is also true for WCS based on small grain cereals and grain legumes. To verify this we conducted a pre-examination using the small dataset where contents of detergent fibre fractions were available. Consideration of contents of aNDF did not yield significant improvements over the equation using proximate constituents (data not shown). Insertion of contents of ADL and ADF showed inconsistent results.

In all approaches e represents the random error term.

$$\text{Approach 1: ME} = \beta + e$$

$$\text{Approach 2: ME} = \beta + \text{starch} + e$$

$$\text{Approach 3: ME} = \beta + \text{ELOS} + e$$

$$\text{Approach 4: ME} = \beta + \text{starch} + \text{ELOS} + e$$

$$\text{Approach 5: ME} = \text{GR} + \beta + e$$

$$\text{Approach 6: ME} = \text{GR} + \beta + \text{GR} \times \beta + e$$

$$\text{Approach 7: ME} = \text{TOG} + \beta + e$$

$$\text{Approach 8: ME} = \text{TOG} + \beta + \text{TOG} \times \beta + e$$

$$\text{Approach 9: ME} = \text{GR} + \text{TOG} + \beta + \text{GR} \times \beta + \text{TOG} \times \beta + e$$

Approaches 2 to 4 had to be conducted using reduced datasets since contents of starch and ELOS were not known for all WCS (starch: $n = 55$; ELOS: $n = 39$). In addition, all approaches were applied using subsets attained by splitting the dataset into WCS composed of cereals ($n = 58$) or peas ($n = 12$) only. Both subsets suffer the same difficulties as the original dataset in terms of uneven distribution of TOG within GR (see Table 1). For use with approaches 2 to 4, the dataset containing only WCS made from cereals had to be reduced due to missing information on contents of starch and ELOS. Only approaches 1 and 3 could be calculated utilising the subset with pea WCS because of the low number of observations.

All statistical evaluations were conducted using least square procedures (SAS, 1988). Partial probabilities – where reported - are based on type III sums of squares (SS); these are each adjusted for all other effects in the model, regardless of order. Generally, probabilities below 0.05 were assumed to be significant unless reported otherwise.

3. Results

The results of estimated ME contents from the current equation are reported in Table 3.

Table 3: Metabolisable energy (ME) concentrations (dry matter (DM) basis) of whole-crop silages as determined in standard digestion trials or estimated from contents of proximate nutrients, and their difference^a

Dataset	n	Mean ME (MJ/kg DM)		Difference ^b (MJ ME/kg DM)	
		Measured (SD)	Estimate ^a (SD)	Mean (SD)	Range
Whole	70	9.89 (0.46)	9.36 (0.29)	-0.53 (0.46)	-1.41 - 0.44
Barley	13	9.85 (0.49)	9.38 (0.24)	-0.47 (0.55)	-1.18 - 0.42
Peas	12	10.16 (0.43)	9.26 (0.25)	-0.90 (0.37)	-1.41 - 0.04
Wheat	25	9.83 (0.34)	9.41 (0.32)	-0.42 (0.37)	-1.11 - 0.24
Triticale	20	9.84 (0.56)	9.36 (0.31)	-0.48 (0.45)	-1.21 - 0.44
East	32	10.05 (0.44)	9.30 (0.21)	-0.75 (0.41)	-1.41 - -0.05
North	26	9.77 (0.37)	9.45 (0.30)	-0.32 (0.38)	-1.06 - 0.44
South	12	9.75 (0.60)	9.35 (0.41)	-0.40 (0.51)	-1.18 - 0.42

SD, standard deviation.

^a ME (MJ/kg DM) = 11.57 – 0.00977 × crude fibre – 0.00711 × crude ash + 0.00621 × crude protein

(Proximate constituents expressed as g/kg DM).

^b Difference = estimated ME – measured ME.

The difference between calculated actual ME was particularly high for silages from eastern Germany and for WCS made from peas. In general, the calculated ME was lower than that derived from digestibility trials for all WCS regardless of GR or TOG. The mean difference for all silages was 0.5 MJ ME/kg DM. We observed a significant influence on the calculated ME of TOG×GR ($P < 0.01$) but neither of GR ($P = 0.28$) nor TOG ($P = 0.23$; data not shown). The difference between calculated and actual ME was significantly influenced by GR ($P < 0.01$) but neither by TOG ($P = 0.21$) nor TOG×GR ($P = 0.30$; data not shown). The actual ME was influenced ($P < 0.01$) by GR and TOG×GR but not by TOG ($P = 0.51$; data not shown).

Table 4 provides information on the effect of TOG, GR and TOG×GR on the chemical composition of the silages.

Table 4: Chemical composition of 70 whole-crop silages as influenced by type of grain (TOG), geographic region of growing (GR) and the combined effect TOG×GR

Proximate constituent	Effect (<i>P</i>) of ^a			<i>r</i> ²	F-Value
	TOG	GR	TOG×GR		
Dry matter			0.29	<0.05	
Ash	<0.05		0.52	<0.05	
Crude fibre			0.43	<0.05	
Crude lipid				NS	
Crude protein	<0.05		0.72	<0.05	
Organic matter	<0.05		0.72	<0.05	
Starch ^b		<0.05	0.51	<0.05	
ELOS ^c	<0.05		0.43	<0.05	

^a Effects were calculated using the GLM procedure of SAS (results from Type III SS).

^b Data on content of starch were available for 55 out of 70 silages.

^c Organic matter solubility in cellulase digestible organic matter in the dry matter; data was available for 39 of 70 silages.

With the exception of crude lipid significant effects of mostly TOG and the interaction effect TOG×GR were visible concerning the proximate constituents of the 70 WCS. If only WCS made from cereals were examined (Table 5), the model became significant only with ash, crude fibre, starch and ELOS as independent variables and merely few significant effects were visible - mostly the interaction effect TOG×GR.

Table 5: Chemical composition of 58 cereal based whole-crop silages as influenced by type of grain (TOG), geographic region of grower (GR) and the combined effect TOG×GR

Proximate constituent	Effect (<i>P</i>) of ^a		<i>r</i> ²	F-Value
	TOG	GR		
Dry matter				NS
Ash			0.41	<0.05
Crude fibre			<0.05	<0.05
Crude lipid				NS
Crude protein				NS
Organic matter				NS
Starch ^b	<0.05		0.50	<0.05
ELOS ^c			0.43	<0.05

^a Effects were calculated using the GLM procedure of SAS (results from Type III SS)

^b Data on content of starch were available for 55 out of 58 silages

^c Organic matter solubility in cellulase digestible organic matter in the dry matter; Data were available for 29 out of 58 silages

The results of the calculations using approaches 1 to 9 are reported in Table 6.

Table 6: Models, coefficients of determination, and significances of the candidate models for estimating the metabolisable energy of whole-crop silages

Approach	Dataset	Model 1		Model 2		n	Degrees of freedom		r ²		SD of residuals		F-Value
		Independent variables (COV)		Model			Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	
		Model	Additional factors	Model									
All silages													
1	All silages	Prox. Const.		ME = COV		70	64		0.26		0.047		
2	content of starch known	Prox. Const.	Starch (COV)	ME = COV		55	49	48	0.39	0.40	0.045	0.045	> 0.100
3	content of ELOS known	Prox. Const.	ELOS (COV)	ME = COV		39	33	32	0.49	0.61	0.050	0.044	< 0.010
4	contents of starch, ELOS known	Prox. Const.	Starch (COV), ELOS (COV)	ME = COV		28	22	20	0.53	0.69	0.053	0.043	< 0.050
5	All silages	Prox. Const.	GR (CLF)	ME = COV		70	64	62	0.26	0.40	0.047	0.043	< 0.010
6	All silages	Prox. Const.	TOG (CLF)	ME = COV		70	64	61	0.26	0.40	0.047	0.043	< 0.010
7	All silages	Prox. Const.	TOG (CLF)	ME = COV		70	64	46	0.26	0.59	0.047	0.035	< 0.050
8	All silages	Prox. Const.	TOG (CLF)	ME = COV		70	64	31	0.26	0.94	0.047	0.014	< 0.001
9	All silages	Prox. Const.	GR (CLF) and TOG (CLF)	ME = COV		70	52	31	0.62	0.94	0.034	0.014	< 0.001
Approach 6 versus 9													
Approach 8 versus 9													
Subset: Silages made from cereals													
1	All silages	Prox. Const.		ME = COV		58	52		0.38		0.046		
2	content of starch known	Prox. Const.	Starch (COV)	ME = COV		55	49	48	0.39	0.40	0.045	0.045	> 0.100
3	content of ELOS known	Prox. Const.	ELOS (COV)	ME = COV		29	23	22	0.55	0.71	0.052	0.042	< 0.010
4	contents of starch, ELOS known	Prox. Const.	Starch (COV), ELOS (COV)	ME = COV		28	22	20	0.53	0.69	0.053	0.043	< 0.050
5	All silages	Prox. Const.	GR (CLF)	ME = COV		58	52	50	0.38	0.43	0.046	0.044	> 0.100
6	All silages	Prox. Const.	GR (CLF)	ME = COV		58	52	40	0.38	0.67	0.046	0.034	< 0.010
7	All silages	Prox. Const.	TOG (CLF)	ME = COV		58	52	50	0.38	0.41	0.046	0.045	> 0.100
8	All silages	Prox. Const.	TOG (CLF)	ME = COV		58	52	40	0.38	0.58	0.046	0.038	> 0.100
9	All silages	Prox. Const.	GR (CLF) and TOG (CLF)	ME = COV		58	52	26	0.38	0.94	0.046	0.014	< 0.001
Approach 6 versus 9													
Approach 8 versus 9													
Subset: Silages made from peas													
1*	All silages	Prox. Const.		ME = COV		10							
2*	content of starch known	Prox. Const.	Starch (COV)	ME = COV		1							
3*	content of ELOS known	Prox. Const.	ELOS (COV)	ME = COV		10							

* These models did not become significant at all (F-value above 0.05).

Abbreviations: ELOS, organic matter solubility in cellulase; COV, covariables: independent variables such as proximate constituents (Prox. Const.), dry matter, starch and organic matter solubility in cellulose, CLF, classification factor; DF, degrees of freedom; F-value, probability of significant improvements of model 2 over model 1; ME, metabolisable energy; GR, geographic region of growing; SD, standard deviation; TOG, type of grain.

The overall accuracy of prediction was low, if only proximate constituents were considered (approach 1) but better, if silages made from peas were not included in this calculation. On examination of the whole dataset, consideration of starch content showed no significant improvements, whereas ELOS did (approach 2 to 4). Also, insertion of GR and TOG (approach 5 and 8) allowed a more accurate prediction and this especially was true when both classification factors were simultaneously considered (approach 9). If only cereal-based WCS were used, the model containing ELOS yielded significant improvements (approach 3) over approach 1. Compared with the whole dataset, consideration of TOG did not offer significant improvements, whereas this was again the case with GR (only approach 6).

4. Discussion

Under practical circumstances it is essential to estimate the feeding value of feedstuffs by rapid and reliable methods. The measurement of contents of proximate constituents of WCS is easy to conduct by standardised procedures. Only, the accuracy of prediction of the energy value for ruminants using contents of proximate constituents (Südekum and Arndt, 1998) remains poor to date. Therefore, this study addressed (I) a validation of the current prediction (Südekum and Arndt, 1998) utilising data from newer digestibility trials on WCS and (II) to look for improvements in estimating the energy density based on contents of proximate constituents and additional constituents of WCS.

On average, the DM contents and chemical composition of the silages used for this study were heterogeneous and the range of values was in good agreement with earlier reports (see review by Südekum and Arndt, 1998). Data of WCS in our dataset were mainly based on crops grown in three different regions and from four different grains (small grain cereals: barley, triticale and wheat; grain legume; pea). In general, mean ME content of all WCS in our dataset as predicted from ash, crude fibre and crude protein concentrations underestimated the actual value derived from standard digestibility trials by about 0.5 MJ ME per kg DM. Since the dataset and the samples used to establish the equation currently in use was no more available, interpretation of our results in comparison with the previous predictions (Südekum and Arndt, 1998) comprises some speculation. We assume, however, that the magnitude of the underestimation of energy density especially of WCS from eastern Germany and of WCS made from peas was severer than that for other WCS sources; the reasons for that remain unclear. Hence, the accuracy of the current prediction is generally poor and even more so for silages made from peas or originating from eastern Germany. Conse-

quently, we tried to improve the prediction. Either additional factors other than the chemical composition or other analytical procedures delivering information more adequate for the estimation of ME content of WCS appear possible. In our dataset, results from the classical "Weende", or proximate constituent, analysis and data on TOG and GR were available for all materials, and contents of starch and ELOS in subsets. In a first step starch and ELOS were examined as potential additional factors. These variables are feasible and their insertion does not inhibit a general equation for all WCS for advisory service use. Contrary, insertion of TOG and GR, whose consideration was examined secondly, would require separate equations and detailed knowledge of the source and origin of WCS and therefore, these variables appear less attractive for laboratory and advisory practise because false mapping or allocation of samples is very likely under routine situations in farming, advisory service and laboratory practise.

We used a statistical model in which each effect was adjusted for all other effects in the model. Hence, strongly aliased effects would not become significant but those being significant can then be accounted for sure. Still, the interpretation of the examined effects concerning TOG or GR needs to be considered with care because only limited data was available and the distribution of TOG and GR within the dataset was unbalanced. Therefore, effects can be confounded and thus being not fully distinguishable.

The prediction of the energy density from the proximate constituents (ash, crude fibre, crude lipid and crude protein) and DM using the complete dataset resulted in a very poor coefficient of determination below 0.30. This indicates that contents of proximate nutrients alone do not deliver good estimates which confirms earlier results (Südekum and Arndt, 1998) and is consistent with the results of other researchers regarding the predictability of concentrations of digestible organic matter in the DM from chemical composition using wheat-based (Adesogan et al., 1998) or maize-based based WCS (Givens et al., 1995).

Surprisingly, insertion of starch as additional factor did not yield significant improvements of the accuracy of the prediction. This seems somewhat surprising since starch - besides the digestible portion of the cell wall - is accountable for most of the energy that can be derived from WCS in the gastro-intestinal tract of ruminants. However, similar observations were reported by others for small grain cereal WCS (Südekum and Arndt, 1998) and maize-based silages (Givens et al., 1995).

Insertion of ELOS as a predictive factor proved to be beneficial with the whole dataset and the subset containing only cereal grain-based WCS. Presumably, consideration of ELOS improved the prediction due to delivering additional information on overall digestibility. Givens et al. (1995) have reported that substantial variations of the proportion of cell wall components and starch were not closely related to differences in digestibility of maize silages. Unfortunately, in this study starch contents and/or ELOS only were available for data subsets. Using these subsets for the prediction of the energy density from contents of proximate constituents alone resulted in higher coefficients of determination than predictions with based on the whole dataset. Hence, data in the subsets may have been more homogenous than that in the whole dataset and results regarding these subsets have to be considered with care. Albeit, our data would still indicate that use of ELOS values of WCS may improve the accuracy of prediction of ME of WCS from laboratory-based measurements, whereas inclusion of starch content appears to be worthless.

Because our dataset contained data from different types of WCS and different regions in Germany, it appeared worthwhile to examine TOG and GR as additional classification factors in the statistical analysis. Data in Table 4 and 5 revealed effects of TOG and GR and the interaction effect TOG \times GR on the chemical composition of the silages for the whole dataset, whereas, for cereal-based WCS, TOG failed to exert an effect. Moreover, a comparison of chemical composition of cereal-based and pea-based WCS indicated considerable differences for crude lipid, crude protein and organic matter concentrations (data not shown). Therefore, we assume that the relationship between chemical composition and energy density may differ as well. This assumption is confirmed by results given in Table 6. By and large, insertion of TOG or GR or both yielded significant improvements of the accuracy of the prediction using the whole dataset, whereas this was only achieved in some cases for cereal-based WCS. Hence, if the energy density is to be estimated from concentrations of proximate constituents, we would strongly advise to regard WCS made from peas and cereals as different entities. Following this recommendation, approaches using the whole dataset will not be considered furthermore here. Instead, in the following we evaluate if it is feasible to establish a general equation at least for all cereal based WCS.

Heeding the dataset containing WCS made from cereals, only the approach with GR and its interactions with proximate constituents yielded significant improvements over the basic approach. This may be reasonably explained by differences in growth conditions of the crops. But then, other small differences might sum up as well, for instance caused by analytic variations. Also the uneven distribution of TOG within GR and vice versa may have biased our results. Hypothetically, consideration of GR in our dataset may at least explain a small proportion of variance that originally was caused by TOG. Insertion of TOG alone or in combination with interactions did not deliver significant improvements. This is consistent with Givens et al. (1989) who found no significant effects of TOG on the energy density of cereal straws. Simultaneous consideration of TOG, GR and their interactions with covariables yielded the highest accuracy ($r^2 = 0.94$) and therefore appears most appropriate. However, this approach encompassed 9 class levels (3 TOG and 3 GR) distributed within 58 cereal-based silages. Hence, variance caused by factors not included in this approach such as between-year differences might be aliased by this model.

Separate equations in terms of TOG and GR would obviously help to achieve the best prediction of ME concentrations using proximate constituents as basic independent variables. Since this may not be feasible for advisory services because additional efforts would be required or a lack of information exists, at least WCS based on cereals and peas should be regarded as different entities and in addition, single equations separated by GR appear promising. Hypothetically, other type of laboratory assays might deliver improved accuracy of the prediction of ME contents of WCS and may also not require separate equations for different regions or type of grains. Further investigations appear however necessary to test our hypotheses. Since a general prediction equation for all silages independent of TOG and GR and deduced from our dataset possesses a coefficient of determination less than that of the equation used up to date (Südekum and Arndt, 1998) and because that prediction is based on a larger dataset than that in the current study, its further application is still recommended.

5. Conclusion

Data available for the evaluation of the predictability of the energy density of WCS showed that a precise estimate of ME from DM and proximate constituents is improved by when type of grain and geographic region of growing are considered. Starch concentration as additional independent variable did not offer enhancements while consideration of ELOS contents appeared promising. Further research appears necessary because available data were biased

in matters of TOG and GR and thus do not allow to separate these effects clearly. Validation of the prediction presently used in practise showed that with our dataset this equation underestimates the energy density by an average of about 0.5 MJ ME per kg DM. Nevertheless, its further use is still recommended for all WCS regardless of which GR and TOG until more detailed knowledge on factors influencing the energy density of WCS may become available.

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Chapter V

General conclusions

1. General conclusions

Improving the efficiency of nutrient utilisation requires precise knowledge on nutrient supply and nutrient requirements of dairy cows. Nutrient utilisation efficiency refers to whole-tract digestibility and absorption. Although the small intestine represents the main site of absorption for most nutrients, the rumen represents a key position in dairy cow nutrition due to vast nutrient transformations occurring in the fore-stomachs. The largest proportion of crude protein at the duodenum originates from rumen microbial degradation and subsequent rumen microbial synthesis, making the assessment of rumen fermentation an essential task in dairy cow nutrition. To reliably assess total supply of crude protein available for digestion in small intestine, quantitative approaches to estimate rumen microbial crude protein synthesis are necessary. However, overall nutrient utilisation efficiency in dairy cow nutrition is the efficiency with which nutrients are used for milk production. Thus, precise knowledge on nutrient requirements of dairy cows is essential as well in order to adjust nutrient supply.

The studies presented in this work deal with both aspects – nutrient requirements and supply. The adequacy of urinary allantoin excretion as a marker for rumen microbial crude protein was examined in the first study. This approach represents a promising non-invasive method to quantify crude protein supply originating from rumen fermentation. A related issue was addressed in the third study but with a view orientated more on feedstuff characteristics and their influence on energy density of a feedstuff which nutrients are predominantly digested in the rumen. Requirements for methionine as an essential amino acid and a new approach postulating a demand for methyl groups were examined in the second study.

The first experiment represents a contribution toward establishing urinary allantoin excretion as adequate quantitative approach to assess ruminal microbial crude protein synthesis. Although further research is necessary to improve its adequacy, this method is a promising tool that is applicable under research and production conditions. The study presented showed that this method under research conditions currently allows semi-quantitative estimates of rumen microbial crude protein synthesis – a ranking of diets and the magnitude of changes between diets in microbial crude protein synthesis were reflected. Therefore, our data augment the applicability of this approach over the more pessimistic view of Shingfield (2000) who concluded from his comprehensive review that this approach only allows a ranking of diets. Because the steers used in our experiments were in the same physiological status

presumably offers a good explanation for our promising results. However, more research appears to be necessary to achieve more precise data using this method.

Generally, this approach represents a promising alternative: Contrary to other available methods and models predicting rumen microbial crude protein synthesis mainly from diet composition, urinary excreted allantoin is a variable directly connected to rumen fermentation. In the light of the vast nutrient transformations occurring in the rumen and the magnitude of factors influencing rumen fermentation, rumen microbial crude protein synthesis is difficult to predict: This assumption is supported by the third study presented in this thesis. Fibre fractions and starch as the main energy delivering components in whole-crop silage were not sufficient to adequately estimate the energy density of whole-crop silages. Since fibre components and starch are fermented predominantly in the rumen it can be assumed that the amount of ruminally synthesised microbial crude protein was not sufficiently explained by the chemical composition of the silages. Similarly, Gosselink et al. (2003) reported that the chemical composition of forages is a poor indication of the duodenal flow of microbial crude protein in ruminants fed all-forage diets. Other workers came to similar conclusions. Bateman et al., (2001a, b) conducted a comparison of several computer models to predict passage of crude protein and amino acids to the duodenum of dairy cows. These authors reported that the predicted passage of nitrogen fractions to the duodenum did not coincide with measured passage at a large percentage of time and they conclude that the models failed to adequately measure the underlying biology of feed changes (Bateman et al., 2001a). As possible reasons, inadequate equations for predicting microbial protein synthesis and feed protein degradation in the rumen and inadequate handling of associative effects of feeds by the models are discussed (Bateman et al., 2001b). Although all models were sufficiently accurate for use in on-farm ration formulation (Bateman et al., 2001b), improving the accuracy of estimates of nutrient flow are central to reducing errors in the prediction of nutrient supply and by this optimising the efficiency of nutrient utilisation (Ahvenjärvi et al., 2003) – both, under research and production conditions. Under research conditions the approach based on urinary purine derivatives has the advantage of being non-invasive. To sample digesta flow reaching the duodenum for digestion, classic marker methods need abomasally or duodenally cannulated animals requiring excessive intervention. Also, the omasal sampling technique developed by Huhtanen et al. (1997) requires cannulated animals although ruminally cannulated animals are sufficient. Contrary, urinary purine derivatives are a non-invasive method controlling efficiency of microbial crude protein production in vivo. Under production conditions, this approach would also be applicable once its adequacy has been improved (Shingfield, 2000). As a main advantage of this approach the prediction of rumen microbial crude protein synthesis it is not based on diet composition, feeding level and other

factors but on excreted metabolites originating mainly from purine bases synthesised in the rumen. Provided that future research can improve the accuracy of the method, its establishment will become a valuable tool to control rumen microbial crude protein production in vivo permitting reliable estimates of crude protein supply to the dairy cow under research and production conditions.

The second study reported in this thesis focused on methionine requirements of dairy cows as one of the most limiting amino acids. Although rumen fermentation represents a key position in dairy cow nutrition regarding nutrient supply, the requirements of the cow are the key criterion and have to be met adequately. In order to improve the efficiency of nutrient utilisation, precise knowledge on the requirements of the host cow is essential. Concerning demand of dairy cows for a specific amino acid pattern, several approaches for determining the daily requirements for amino acids exist: factorial approaches like the Cornell net carbohydrate and protein system (CNCPS; O'Connor et al., 1993), results from dose-response trials – for instance the concept of the Ideal Protein (Rulquin et al., 1993; Doepel et al., 2004) or simply the milk protein score (Schingoethe, 1996) delivering different numbers for the requirement (Piepenbrink et al., 1998). Also, several amino acids are considered to be limiting. The second study in this thesis is a new approach towards better understanding of the dairy cow's requirement for methionine which along with lysine frequently is proposed as first or second limiting amino acid (Schwab et al., 1992; Pisulewski et al., 1996; Schwab, 1996). Methyl group requirements are proposed as a new figure in dairy cow nutrition and possible interactions with the metabolism of methionine are theoretically derived. Additional research is necessary to validate the interrelationships presumed since approaches of others on the mechanisms and metabolic pathways affecting the effectiveness of supplemental rumen-protected choline appear reasonable as well – e.g., interrelationships with the tetrahydrofolate system have been proposed (Pinotti et al., 2004; Baldi and Pinotti, 2005). However, both approaches assume an elevated demand for methyl groups during the transition period, especially at the beginning of the lactation. Provided that this reflects the true situation in dairy cows, consideration of methyl group requirements of dairy cows may remedy a potential limiting factor. Supplying methyl groups in a form that becomes available at the small intestine instead of being degraded in the rumen, may increase overall efficiency of nutrient utilisation by increasing yield as already shown in several experiments (for review see Pinotti et al., 2003). Hence, rumen-protected choline represents another selective supplementation that serves to overcome single nutrient limitations and leading to an improved overall nutrient utilisation efficiency.

In conclusion, two starting points in improving nutrient utilisation efficiency in dairy cows have been derived in this thesis. Rumen fermentation as an indirect variable and interactions between choline and methionine metabolism as direct variables have been considered. The studies reported in this thesis deliver two valuable approaches for future research aiming at improvements of overall nutrient utilisation efficiency in dairy cows. Although the method up to date lacks precision, urinary allantoin excretion represents a promising non-invasive tool potentially allowing control of rumen microbial crude protein production *in vivo*. The necessity for this was nicely shown using the predictability of the energy density of whole-crop silage. Also, considering potential interactions between methionine and choline metabolism at the level of interchangeable methyl groups appears as a promising approach in refining requirements for methionine of dairy cows. Thus, adequacy in diet formulation may be improved as will the efficiency of nutrient utilisation.

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