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Efficiency of endogenous urea ^{15}N nitrogen incorporation into bacterial and milk protein of goats fed diets with three different protein levels*

J.P. Michalski[1,3], J. Kowalczyk[1], J. Voigt[2], H.M. Hammon[2], M. Czauderna[1] and C.C. Metges[2]

[1]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences
05-110 Jabłonna, Poland
[2] Research Unit Nutritional Physiology,
'Oskar Kellner' of the Leibniz Institute for Farm Animal Biology
18196 Dummerstorf, Germany

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ABSTRACT

The aim of the study was to estimate the magnitude of endogenous blood urea nitrogen (EBUN) incorporation into bacterial protein produced in the rumen and into milk protein of goats fed low (LP), medium (MP) or high protein (HP) diets arranged in a 3 x 3 Latin Square design. Three Alpine goats of about 35 kg body weight fitted with cannula into the rumen and a catheter into the jugular vein were fed three isoenergetic diets containing 11(LP), 13(MP) or 16(HP)% crude protein in dry matter (DM). Goats were continuously infused ^{15}N urea into the jugular vein for 6 days. Daily milk yield was 1575, 1492 and 1770 g. Concentrations of milk urea were 139, 342 and 451 mg/l, whereas plasma urea was 178, 356 and 667 mg/l in LP, MP and HP groups, respectively. ^{15}N excess in urinary N was higher in the HP group, and in faecal N was higher in the LP group, respectively ($P<0.05$). In rumen bacteria mass enrichment of ^{15}N (atom% excess) decreased when the level of ammonia nitrogen in the rumen fluid increased with the level of protein in the diet. These values indicate a most efficient utilization of recycled urea nitrogen in the rumen for bacterial protein synthesis in goats consuming the diet with the lowest protein level. ^{15}N excess in milk urea differed ($P<0.05$) among groups (0.36, 0.74 and 1.22 % of ^{15}N dose in LP, MP, and HP groups, respectively), indicating dependence on the level of protein in the diet. The highest ($P<0.05$) amount of infused ^{15}N accumulated in the milk protein of the group LP (6.88, 4.50 and 2.23 % of ^{15}N dose in group LP, MP and HP, respectively).

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[3] Corresponding author: e-mail: j.p.michalski@op.pl

The degree of ^{15}N incorporation into milk protein was positively correlated to ^{15}N enrichment in bacteria (r=0.934) indicating that endogenous urea nitrogen was incorporated into bacterial protein in the rumen and afterwards consequently into the amino acids of milk protein as a main pathway of endogenous urea nitrogen incorporation into milk protein.

It is concluded that in lactating goats about 50% of the ruminal bacterial N requirement can be supplied by the rumino-hepatic cycle without reduction of milk yield.

KEY WORDS: endogenous urea, ^{15}N, rumen bacteria, milk protein, protein level, dairy goats

INTRODUCTION

In ruminant animals, significant amounts of non-protein nitrogen compounds such as ammonia, circulates between the digestive tract and the blood (Houpt, 1959; Gärtner et al., 1961). A substantial amount of this ammonia is converted in the liver to endogenous urea, which can be transferred to the rumen (Kowalczyk et al., 1975a,b; Harmeyer and Martens, 1980; Kennedy and Milligan, 1980). Endogenous urea is hydrolysed in the rumen (Gibbons and McKarthy, 1957) and the resultant ammonia enters the ruminal ammonia pool (Bunting et al., 1989). This ammonia is utilized by rumen bacteria, as an important source of nitrogen for bacterial protein synthesis (Virtanen, 1964). This nitrogen is incorporated at various proportions in different amino acids of bacterial protein and the proportions depend on dietary composition (Havassy et al., 1982).

The amount of urea transferred to the rumen and its utilization for bacterial protein synthesis depends on numerous factors. The most important is the amount of nitrogen compounds, carbohydrates, and types of fibre in the diet and rates of synchronization of its degradation in the rumen (Kennedy and Milligan, 1980; Kowalczyk et al., 1982; Lapierre and Lobley, 2001).

It was shown in previous work (Kowalczyk et al., 1975a), that in ruminants, the level of protein in the diets is an important factor regulating the retention of endogenous urea nitrogen and the excretion of endogenous urea in the urine and faeces. In sheep, the amount of endogenous nitrogen utilized for the synthesis of bacterial protein can exceed half of the dietary nitrogen when the supply is insufficient (Kowalczyk et al., 1975a,b). In growing bulls, up to 16% of the synthesized ruminal microbial N could be covered by recycled endogenous N without decreasing N retention (Kluth et al., 2000). The transfer of blood urea into the forestomachs is controlled not only by dietary factors but also by the physiological state of the animal, as growth, pregnancy and lactation. Urea excretion in the urine in lactating goats was lower than during the dry period (Brun-Bellut, 1996; Ndibualonyi et al., 1998), which tended to occurre at both low and high levels of protein in the diet. Rumen recycled urea varied with physiological state being higher for lactation and lower during drying period in sheep

(Brun-Bellut, 1996). Changes in urea recycling due to the level of dietary protein, pregnancy, lactation or growth has been documented and comprehensive reviews have been presented (Harmeyer and Martens, 1980; Kennedy and Milligan, 1980; Lapierre and Lobley, 2001). However, there are only few data on the utilization of blood urea nitrogen during lactation (Brun-Bellut, 1996; Al-Dehneh et al., 1997), especially the influence of the protein level in the diet on endogenous urea nitrogen incorporation into milk urea and milk protein (Pfeffer et al., 2009).

The aim of the study was to estimate the magnitude of endogenous blood urea nitrogen incorporation into rumen bacteria protein and into milk protein in goats fed low, medium or high protein level in the diet.

MATERIAL AND METHODS

Animals and nutrition

The experiment was carried out in three primiparous Alpine goats at 105 days of lactation. In the second month of lactation each goat was surgically equipped with a soft rubber rumen cannula, with a bore diameter of 25 mm, in accordance with generally accepted principles. The day before the infusion, goats were permanently fitted with a polyurethane catheter into the jugular vein (16G x 200 mm). The experimental design was 3 x 3 Latin Square. Lactating goats were 12 months old and weighed 35±2 kg. They were housed in individual cages and fed every 6 h 550 g of one of three isoenergetic diets containing, low (LP), medium (MP) or high (HP) levels of protein, respectively. The crude protein concentration was 11%, 13% and 16% in dry matter (DM), respectively. The total daily amount of the diet (1200 g hay and 1000 g concentrate), was divided into four portions (4 x 550 g). Water was freely available. Concentrate composition is given in Table 1 and nutrient contents are given in Table 2.

Table 1. Composition of concentrate, %

Compounds	Protein level in diets		
	low	medium	high
Barley grain	11.56	11.51	11.63
Dried beet pulp	40.13	40.03	40.44
Wheat starch	35.88	28.81	13.55
Soyabean meal	10.43	17.65	32.36
Mineral-vitamin-mix[1]	2.01	2.00	2.02

[1] Polfamix OK (BASF)

Table 2. Nutrient content of feeds, % in dry matter

Indices	Hay	Concentrate, protein level		
		low	medium	high
Dry matter	91.53	89.21	89.68	89.68
Crude protein	11.27	11.07	15.05	21.60
Ether extract	2.15	0.95	1.65	2.21
Crude fibre	30.66	8.51	8.40	8.08
Ash	5.95	4.24	4.72	5.54
Metabolizable energy, MJ/kg	10.55	12.58	12.56	12.44

Experimental design and sampling

The 22-days experimental period consisted of 14 days of adaptation to the diets and 8 days of sample collection. After the adaptation period, samples of feed, milk, rumen liquid, blood, urine and faeces were collected daily for respective analyses.

The refusals were weighed and pooled in a collective sample for analysis. For the collection of faeces and urine, animals were placed in cages equipped with a perforated sheet on the floor, enabling the separation of urine from faeces. Faeces were collected immediately after excretion, pooled and stored at 4°C. The daily amount of faeces was weighed and samples of appropriate amounts were stored at -20°C. Urine was collected in containers with 60 ml of 6N HCl. The daily amount of urine was weighed and appropriate samples were stored at -20°C.

Goats were milked twice a day at 06.00 and 18.00 h; milk was weighed and pooled for 24 h periods. Rumen liquid (200 ml) and blood samples from jugular vein were collected twice a day at 08.00 and 14.00 h. For blood sampling, the i.v. catheter was rinsed with 10 ml of isotonic saline. The first 5 ml of blood samples were discarded. After that, 9 ml blood was collected in heparinised tubes. The tubes were placed on crushed ice until centrifuged at 1450 *g* for 15 min at 4°C for preparation of plasma. Rumen bacteria were isolated from rumen liquid by two-step centrifugation, according to Meyer et al. (1967). Collected samples were stored at -20°C until analyses.

^{15}N-urea infusion

From the second day of sample collection, goats were continuously infused with ^{15}N-labelled urea (VEB Berlin-Chemie, Berlin, Germany) 98 atom % excess; 1.6 g/d $^{15}N_2$-urea, dissolved in 600 ml of 0.9% NaCl solution) into the jugular vein for 6 days to label the metabolic N pool. Amount of ^{15}N administered was approximately 5 mg $^{15}N \cdot kg^{-1}$ body weight (BW) per day.

Analyses

The nutrient content in feeds and total N in urine, faeces and milk were

determined according to AOAC procedures (1990). Ammonia in rumen liquid was determined by microdiffusion (modified after Conway, 1954). The concentrations of urea in milk and blood plasma samples were quantified using high-performance liquid chromatography with *pre*-column derivation (Czauderna and Kowalczyk, 2009). The content of ^{15}N in samples of rumen bacteria, faeces, and urine was determined at the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf (Germany), by means of isotope-ratio mass spectrometry, (IRMS; Delta S, Finnigan MAT, Bremen, Germany) after combustion of the dried samples by an elemental analyzer EA 1108 (Carlo Erba., Milan, Italy). For the determination of ^{15}N in milk protein, the protein fraction of milk was isolated by precipitation with 20% trichloroacetic acid (2:1, v/v) and centrifugation (3000 *g* for 5 min at 4°C). The dried milk protein was used. For analysis of the ^{15}N level in milk urea, 5 ml of milk were incubated with 0.1 mg of urease (9 U/mg) for 3 h at 40°C. The resultant NH_3 was separated by microdiffusion (Voigt and Steger, 1967), taken up in 4 ml of 0.01 M HCl, and was evaporated to dryness (at 60°C, air-forced oven). The ^{15}N enrichment of all samples was measured against a standard gas, which was calibrated against air nitrogen with ammonium sulphate as reference. A sample size of about 20 to 100 μg N was used for combustion (Lahann et al., 2010).

Calculations

The ^{15}N excretions in faeces, urine and milk (g/d) were calculated as the product of N excretion (total N, protein-N, and urea-N, respectively) and ^{15}N at % excess of the faeces, urine and milk according to the following equation:

$$^{15}\text{N excretion (g/d)} = \frac{\text{N (g/d)} \times {}^{15}\text{N (at\%)} \times 15}{1400} \quad (1)$$

Unfortunately, the urea in the blood samples was contaminated with ^{15}N from the infusion solution, thus, it was assumed that ^{15}N enrichment of milk urea was essentially equal to that of urea entering the rumen from blood since urea in milk and that secreted into the rumen has a common source (blood urea). Hence, the estimated atom-%^{15}N-excess of milk urea-N was used for calculation of the proportion of bacterial N synthesized in the rumen originating from endogenous urea.

The proportion of endogenous urea N of ruminal bacterial N was estimated as follows:

$$\text{Endogenous N in bacterial N (\% of total N)} = \frac{^{15}\text{N in bacteria (at\%)}}{^{15}\text{N in milk urea (at\%)}} \times 100 \quad (2)$$

The proportion of the endogenous N in the milk protein-N was calculated analogously.

The amounts of endogenous urea N transferred into milk protein N was estimated as follows:

$$\text{Endogenous N (g/d)} = \frac{^{15}\text{N in milk protein (mg/d)} \times 14}{150 \times ^{15}\text{N in milk urea N (at\%)}} \quad (3)$$

Statistical analysis

The data were statistically analysed using the nonparametric Mann-Whitney U test for comparing independent experimental groups and regression analysis. The Statistica v. 6 package was used (2002; www.statsoft.pl).

Means were considered significantly different at $P<0.05$.

RESULTS AND DISCUSSION

No significant differences in feed intake were found between diets of goats (Table 3).

Table 3. Diet intake, chemical composition, metabolic energy of feed intake and milk yield. Mean values ± SD (n=9)

Indices	Protein level in diets[1]		
	low	medium	high
Diet intake, g DM/d	1654 ±196	1644 ± 226	1782 ± 127
Crude protein, % DM	11.49^{a} ± 0.41	13.27^{b} ± 0.24	17.18^{c} ± 0.42
Ether extract, % DM	1.64^{a} ± 0.08	2.02^{b} ± 0.14	2.31^{c} ± 0.08
Crude fibre, % DM	15.53 ± 2.24	17.35 ± 0.24	16.84 ± 1.02
Ash, % DM	5.24 ± 0.27	5.26 ± 0.33	5.77 ± 0.19
ME, MJ/kg DM	11.92 ± 0.18	11.78 ± 0.02	11.73 ± 0.08
Milk yield, g/day	1575^{a} ± 182	1492^{aA} ± 296	1770^{Bb} ± 229
Milk N, %	0.47^{a} ± 0.04	0.49^{ab} ± 0.02	0.50^{b} ± 0.03

[1] values with different letters differ significantly at ^{a,b} $P<0.05$ and ^{A,B} $P<0.01$ levels, respectively

Milk yield (Table 3) was not correlated with the level of protein in the ration. Other authors observed a small positive correlation between the level of protein in diet and the daily milk yield (Wang et al., 2007; Zhai et al., 2007).

However there was a weak positive correlation (r=0.338) between the protein content in the diets and the total nitrogen content in the milk (Table 3). Positive correlation (r=0.707) was also observed between protein content in the diets and urea in milk (Table 4), which agrees with other investigations (Zhai et al., 2007; Bonanno et al., 2008).

The concentration of urea in milk was lower than in plasma, and these values were positively correlated (r=0.769; Figure 1), which is consistent with the results of Cabiddu et al. (1999) ,Wang et al. (2007) and Zhai et al. (2007). However Bava et al. (2001) reported higher levels of urea in milk than in plasma, but also a positive correlation between these values.

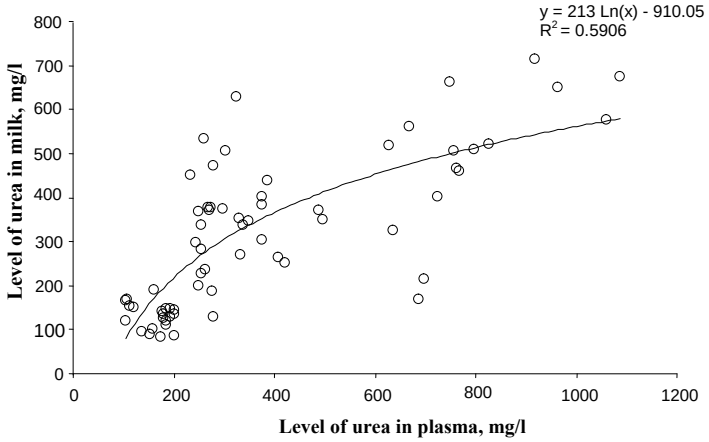

Figure 1. The relationship between the concentration of urea in milk and plasma

The content of ammonia in rumen fluid (Table 4) differed highly between the diets ($P<0.01$). In comparison to low protein feeding, the amount of ruminal ammonia was greater at the medium dietary protein level, and greatest at the high dietary protein level (by 114% and 340%, respectively). The results indicate that the level of protein in the diet significantly affected the concentration of ammonia nitrogen in rumen fluid, probably as a result of a greater amount of protein degradation in the rumen.

Plasma urea concentrations differed significantly between groups ($P<0.01$), and increased together with the level of protein in the diets (Table 4). At the medium level of protein, urea in blood plasma was increased by 100 % and at the high level by 275%.

There were differences among the diets in the concentration of nitrogen in the faeces, but significant differences were only between the HP diet and the other two diets. High protein intake resulted also in higher nitrogen excretion in feaces than after low protein intake (Table 4). Marini and Van Amburgh (2003) observed increased excretion of nitrogen in the faeces when feeding high protein diets in Holstein heifers.

The level of protein in the diet had an impact on nitrogen excretion in the urine (Table 4). At higher levels of protein in the diet nitrogen excretion in the urine increased, respectively, by 69% and 218%. There were no differences in nitrogen content in urine. Increased excretion of nitrogen was due to increased urinary excretion in the animals with higher levels of protein. Urine excretion was 1461, 2494 and 3170 g day^{-1}, respectively.

The increase of the urinary N excretion with growing protein level in the diet corresponded with the excretion of urea (Table 4).The excretion was by 100%

greater at medium level of protein in the diet, and by 352% greater at the highest level of dietary protein (P<0.01). Urea-N as % of total urinary N amounted to 60 (LP), 71 (MP), and 85 (HP), respectively. Recently Pfeffer et al. (2009) found the same influence of the dietary protein level on urinary urea-N in lactating goats.

Table 4. Intake and excretion of N, the level of ammonia in the rumen fluid and of urea in plasma. Mean values ±SD

Indices	Protein level in diets[1]		
	low	medium	high
N intake, g/day	27.1	31.1	43.7
Faecal N, %	$0.67^{A} \pm 0.12$	$0.75^{a} \pm 0.05$	$0.81^{Bb} \pm 0.04$
g/day	11.26 ± 1.71	12.06 ± 2.58	12.06 ± 1.03
Urinary N, %	0.40 ± 0.08	0.42 ± 0.10	0.63 ± 0.27
g/day	$5.66^{A} \pm 0.88$	$9.59^{B} \pm 3.42$	$17.98^{C} \pm 1.57$
Urinary urea, g/day	$7.26^{A} \pm 2.24$	$14.55^{B} \pm 4.58$	$32.82^{C} \pm 7.68$
Milk, g N/day	$7.35^{A} \pm 0.58$	$7.34^{A} \pm 1.29$	$8.76^{B} \pm 1.18$
urea, mg/l	$138.9^{A} \pm 41.9$	$341.9^{Ba} \pm 99.2$	$451.3^{Bb} \pm 156.1$
urea, mg/day	$219.4^{A} \pm 75.5$	$504.1^{B} \pm 150.3$	$814.5^{C} \pm 337.7$
N retention, g/day	2.83 ± 2.77	2.11 ± 1.98	4.90 ± 0.54
NH_3 in rumen liquid, mg/l	$35.0^{A} \pm 8.41$	$74.8^{B} \pm 45.0$	$154.0^{C} \pm 37.5$
Plasma urea, mg/l	$178^{A} \pm 64$	$356^{B} \pm 161$	$667^{C} \pm 251$

[1] values with different letters differ significantly at [a,b] P<0.05 and [A,B] P<0.01 levels, respectively

Eriksson and Valtonen (1982), Faix et al. (1988), Tebot et al. (1998) and Wang et al. (2007), also showed a decrease in the concentration of urea in the urine and quantity of urea excreted, in response to a reduced dietary protein concentration. The amount of urea excreted in the urine was also positively correlated with plasma urea concentrations (r=0.757; Table 4), which corresponds to the results of other authors (Marini and Van Amburgh, 2003; Wang et al., 2007). Thus, the urea clearance (urea in urine [g/d)/plasma urea (g/l)] was nearly constant in all 3 diets (40.8 l/24 h). This means that the excretion function of the kidneys for urea was independent of the protein content of diets.

The nitrogen content in the dry matter of rumen bacteria was 7.1% in the LP diet, and 8.5 % in the MP and HP diets (not shown). Thus, in goats fed a diet of medium and high protein content nitrogen content was 21% higher than in the LP group (P<0.01) ^{15}N-excess in bacterial mass, during the infusion of labeled urea, was greatest in goats treated with the LP diet and lowest in HP goats (Table 5). Statistically significant differences were found between the diet group with high levels of protein and the other diets (P<0.01). So the level of protein in the diet affected the amount of ^{15}N-excess in rumen bacterial mass. This indicates greater utilization of endogenous urea by rumen microorganisms in goats in the protein-restricted diet. As shown in Table 5, the portion of the endogenous N of bacterial N decreases from 53% to 15% if the dietary CP content increases from 11.5% to 17.2% in DM. Greater utilization of urea could have resulted from higher recycling of urea to the rumen during low protein feeding (Kowalczyk et al., 1975a; Kennedy and Milligan, 1980; Brun-Bellut, 1996).

The observed differences in the utilization of endogenous N by rumen bacteria resulted mainly from differences in the concentration of ammonia in the rumen (Table 4). This has been demonstrated many times by other authors (Gärtner et al., 1961; Remond et al., 1996).

The proportion of endogenous N incorporated into milk protein corresponded with the utilization of endogenous N by rumen bacteria (Figure 2). The proportion decreased from 25% to 7% with increasing levels of CP in the diets (Table 5).

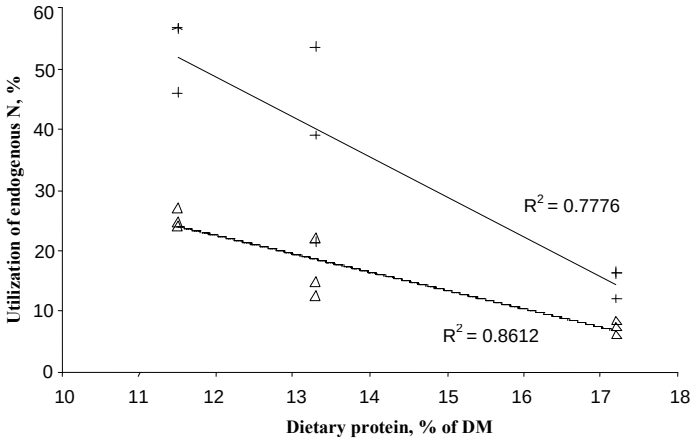

Figure 2. The relationship between dietary protein and utilization of endogenous nitrogen by rumen bacteria (+) and for synthesis of milk protein (Δ) in all periods of infusion

Table 5. ^{15}N (at% excess) in bacterial crude protein, milk urea and milk protein during continuous infusion of labeled urea in all periods of infusion. Mean values ±SD (n= 54)

Indices	Protein level in diets[1]		
	low	medium	high
Bacterial crude protein (CP)	$1.35^{a} \pm 0.69$	$0.92^{a} \pm 0.49$	$0.36^{b} \pm 0.19$
Milk urea	2.55 ± 0.86	2.42 ± 0.78	2.38 ± 0.43
Milk protein	$0.646^{A} \pm 0.221$	$0.403^{B} \pm 0.195$	$0.177^{C} \pm 0.094$
^{15}N bacteria/^{15}N urea	$0.53^{A} \pm 0.38$	$0.38^{A} \pm 0.47$	$0.15^{B} \pm 0.09$
^{15}N milk protein/^{15}N urea	$0.25^{aA} \pm 0.17$	$0.17^{bA} \pm 0.16$	$0.07^{B} \pm 0.04$
^{15}N milk protein/^{15}N bacterial CP	0.48 ± 0.08	0.44 ± 0.14	0.49 ± 0.19

[1] values with different letters differ significantly at ^{a,b} $P<0.05$ and ^{A,B} $P<0.01$ levels, respectively

Most of infused ^{15}N was excreted in the urine, followed by faeces, and the smallest amount was secreted in milk (Table 6). Total excretion in faeces and urine of administered ^{15}N was lowest in the group with the low level of dietary protein and increased with increasing levels of protein in the diets. This indicates increased utilization of endogenous urea nitrogen by goats fed the low-protein diet (Table 6).

In the urine the highest proportion of ^{15}N was excreted in goats fed the high protein diet and the lowest in goats fed low protein diets ($P<0.05$) (Table 6). Higher

excretion of endogenous urea nitrogen in the urine of goats fed a high protein diet may be due to less intensive transfer of urea from the blood into the rumen and thus its increased removal by the kidneys.

In contrast to urine, in faeces the highest ^{15}N excretion was observed in animals fed the low-protein diet ($P<0.05$; Table 6). This could be caused by the increased recycling of endogenous urea into the rumen, and utilization of this nitrogen by microorganisms for protein synthesis followed by incomplete absorption of nitrogen compounds in the gut. Also Kowalczyk et al. (1975a) observed in the faeces, higher level of nitrogen from intravenous urea infusion in animals fed low-protein diet. However, it has also to be considered that the increased urea recycling in the LP diet could be caused not only from the lower protein content in feed, but also due to the higher content of wheat starch in the LP diet (Table 1). This was probably a source of easily available energy for microbial protein synthesis, which also resulted in an increased nitrogen transfer to the rumen (Remond et al., 1996, Al-Dehneh et al., 1997).

Daily secretion of ^{15}N excess in milk was highest in the animals fed the LP diet, and lowest in the animals fed the HP diet (Table 6).

Table 6. ^{15}N-excess and percent of injected ^{15}N in urine, faeces and milk during continuous infusion of labeled urea in all periods of infusion. Mean values ±SD (n=54)

Indices		Protein level in diets[1]		
		low	medium	high
^{15}N infused, g/day		0.701 ± 0.030	0.702 ± 0.034	0.704 ± 0.046
Urine	^{15}N excess, g/day	0.196^{a} ± 0.042	0.284^{a} ± 0.090	0.402^{b} ± 0.037
	% of infused	27.83^{a} ± 5.15	40.69^{ab} ± 13.51	57.24^{b} ± 6.98
Feaces	^{15}N excess, g/day	0.107^{a} ± 0.032	0.062^{b} ± 0.019	0.033^{b} ± 0.015
	% of infused	15.29^{a} ± 4.67	8.77^{b} ± 2.55	4.76^{b} ± 2.29
Milk	^{15}N excess, g/day	0.051^{A} ± 0.019	0.037^{AB} ± 0.020	0.024^{B} ± 0.007
	% of infused	7.31^{A} ± 2.81	5.22^{AB} ± 2.89	3.46^{B} ± 1.12

[1] values with different letters differ significantly at ^{a,b} $P<0.05$ and ^{A,B} $P<0.01$ levels, respectively

In milk urea, 0.36% to 1.22% of the administered ^{15}N was secreted (Table 7). The largest amount was secreted in the goats fed the HP diet and the lowest in goats fed LP diet ($P<0.05$). The level of protein in the ration influenced the ^{15}N-incorporation into milk protein ($P<0.01$; Table 5), and the amount of ^{15}N secreted daily in milk protein ($P<0.05$; Table 7). From 2.2% to 6.9% of the administered urea nitrogen was secreted in the milk protein. Administered ^{15}N was incorporated most intensively in the milk protein of goats fed LP diet. Goats fed this diet utilized endogenous urea nitrogen to a greater degree for milk protein synthesis than the animals fed the high protein diet, due to the economical use of nitrogen compounds.

The daily amount of ^{15}N incorporated into milk protein was negatively correlated ($r = -0.456$; $P<0.05$) with the amount of ^{15}N secreted into milk as urea (not shown),

so it can be concluded that the utilization of endogenous urea nitrogen for milk protein synthesis is negatively correlated with the amount of endogenous urea secreted into milk (Table 7).

Table 7. ^{15}N-excess secreted in milk urea and milk protein and percent of the total dose ^{15}N in milk urea and milk protein during all periods of infusion. Mean values ±SD (n=9)

Indices		Protein level in diets[1]		
		low	medium	high
Milk urea	^{15}N excess, g	$0.015^{a} \pm 0.010$	$0.031^{ab} \pm 0.015$	$0.051^{b} \pm 0.011$
	% of ^{15}N dose	$0.36^{a} \pm 0.24$	$0.74^{ab} \pm 0.35$	$1.22^{b} \pm 0.27$
Milk protein	^{15}N excess, g	$0.290^{a} \pm 0.035$	$0.189^{a} \pm 0.089$	$0.094^{b} \pm 0.012$
	% of ^{15}N dose	$6.88^{a} \pm 0.82$	$4.50^{a} \pm 2.12$	$2.23^{b} \pm 0.28$

[1] values with different letters differ significantly at ^{a,b} $P<0.05$

It was a very high positive correlation (r=0.934) between the amount of ^{15}N-incorporated into rumen bacteria and in milk protein (Figure 3), which indicates the utilization of amino acids originating from bacterial protein for milk protein synthesis. Thus, utilization of nitrogen from endogenous urea for milk protein synthesis depends on the transfer of nitrogen to the rumen and the use of this nitrogen by rumen bacteria. After the data in Table 5 (last line) the bacterial crude protein (CP) was utilized from 44 to 49% for the synthesis of milk protein. An influence of the protein level in the diet is not to be recognized.

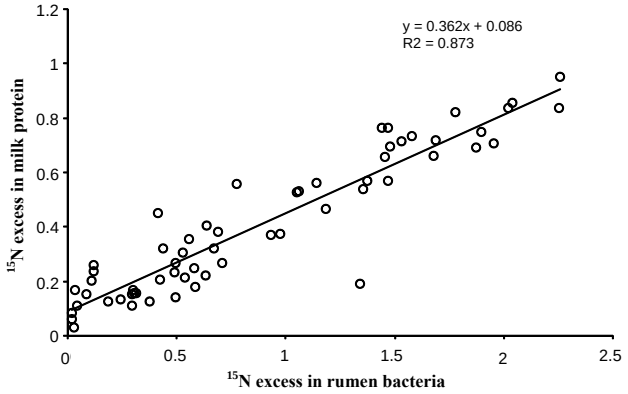

Figure 3. The relationship between incorporation of ^{15}N into the bacterial and milk protein, atom % excess

CONCLUSIONS

The present study confirms that endogenous urea nitrogen can be used by lactating goats for synthesis of bacterial and milk protein. Increasing the protein

content in the diet reduces the efficiency of endogenous urea nitrogen utilization. In a feeding period of 22 days with a dietary crude protein level of 115 g·kg DM^{-1} and an energy level of 11.9 MJ ME·kg dry matter $(DM)^{-1}$, about 50% of the bacterial N demand were supplied by the rumino-hepatic cycle without reduction of milk yield and N-retentionin comparison to an isoenergetic diet with 133 g crude protein·kg DM^{-1}. Consequently, for the N balance in lactating goats (105 days in milk, about 45 g of milk·kg BW^{-1}) the ruminal N balance (N-intake - N-outflow from the rumen to the intestine by undigested protein and microbial N) can be negative and reach a level of 50% of the ruminal microbial N requirements. This value is far higher than earlier found in growing bulls (Kluth et al., 2000). That means that the utilization of the endogenous urea is more efficient by the lactating goat in comparison to the growing cattle. Pfeffer et al. (2009) found in lactating goats in comparison to lactating milk cows a higher utilization rate of endogenous urea degraded in the digestive tract. On the other hand, long time investigations are necessary to verify the high potential of the use of endogenous N by rumen microbes in high-yielding lactating goats fed protein-low diets. The reason for the higher milk yield and N-retention in goats fed the high protein diet is presumably a result of the specific effect of the higher amino acid availability in goats.

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REFERENCES

Al-Dehneh A., Huber J.T., Wadeley R., Theurer C.B., Pessarakli M., de Young D., 1997. Incorporated of recycled urea-N into ruminal bacteria flowing to the small intestine of dairy cows fed a high-grain or high-forage diet. Anim. Feed Sci. Tech. 68, 327-338

AOAC, 1990. Association of Official Analytical Chemists, Official Methods of Analysis. 15th Edition. Washington, DC

Bava L., Rapetti L., Crovetto M., Tmburini A., Sandrucci A., Galassi G., Succi G., 2001. Effects of nonforage diet on milk production, energy, and nitrogen metabolism in dairy goats throughout lactation. J. Dairy Sci. 84, 2450-2459

Bonanno A., Todaro M., Di Grigoli A., Scatessa M.L., Tornambe G., Alicata M.L., 2008. Relationships between dietary factors and milk urea nitrogen level in goats grazing herbaceous pasture. Ital. J. Anim. Sci. 7, 219-235

Brun-Bellut J., 1996. Urea recycling in the rumen of dairy goats: effects of physiological stage and composition of intake. Small Ruminants Res. 23, 83-90

Bunting L.D., Boling J.A., MacKown C.T., Davenport G.M., 1989. Effect of dietary protein level on nitrogen metabolism in the growing bovine: I Nitrogen recycling and intestinal protein supply in calves. J. Anim. Sci. 67, 820-826

Cabiddu A., Branca A., Decandia M., Pes A., Santucci P.M., Masoero F., Calmari L., 1999. Relationship between body condition score, metabolic profile, milk yield and composition in goats browsing a Mediterranean scrubland. Livest. Prod. Sci. 61, 267-273

Conway E.J., 1954. Micro Diffusion Analysis and Volumetric Error. Crosby, Lockwood and Son Ltd. London

Czauderna M., Kowalczyk J., 2009. Easy and accurate determination of urea in milk, blood plasma, urine and selected diets of mammals by high-performance liquid chromatography with photodiode array detection preceded by pre-column derivatization. Chem. Anal. (Warsaw) 54, 919-937

Eriksson L., Valtonen M., 1982. Renal urea handling in goats fed high and low protein diets. J. Dairy Sci. 65, 385-389

Faix S., Leng L., Szanyiová M., Boďa K., 1988. Effects of dietary energy intake on tubular reabsorption of urea in sheep. Physiol. Bohemoslovaca 37, 493-501

Gärtner K., Decker P., Hill H., 1961. Untersuchungen über die Passage von Harnstoff und Ammoniak durch die Pansenwand von Ziege. Pflügers Arch. 274, 281-288

Gibbons R.J., McKarthy R.D., 1957. Obligatory anaerobic urea-hydrolyzing bacteria in the bovine rumen. Maryland Agric. Exp. Sta. Misc. Publ. 291, 12-16

Harmeyer J., Martens H., 1980. Aspects of urea metabolism in ruminants with reference to the goat. J. Dairy Sci. 63, 1707-1728

Havassy I., Kowalczyk J., Horsky K., Kosta K., Otwinowska A., 1982. Incorporation of ^{15}N – urea into individual amino acids of rumen bacteria and blond plasma protein in sheep. Acta Physiol. Pol. 33, 415-419

Houpt T.R., 1959. Utilization of blood urea in ruminants. Amer. J. Physiol. 197, 115-120

Kennedy P.M., Milligan L.P., 1978. The effects of cold exposure on digestion, microbial synthesis and nitrogen transformation in sheep. Brit. J. Nutr. 39, 105-117

Kennedy P.M., Milligan L.P., 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: A review. Can. J. Anim. Sci. 60, 205-221

Kluth H., Gabel M., Voigt J., Schönhusen U., 2000. The use of endogenous nitrogen for microbial crude protein synthesis in the rumen of growing bulls. J. Anim. Physiol. Anim. Nutr. 84, 136-147

Kowalczyk J., Havassy I., Košťa K., Otwinowska A., 1975b. Incorporation of intravenously administered ^{15}N labeled urea in to the bacterial protein in the sheep. Acta Physiol. Pol. 26, 307-312

Kowalczyk J., Havassy I., Otwinowska A., Košťa K., 1975a. Passage of intravenously administered ^{15}N urea into the digestive tract and its excretion in sheep. Acta Physiol. Pol. 26, 299-306

Kowalczyk J., Havassy I., Otwinowska A., Košťa K., 1982. Utilization of urea ^{15}N for the synthesis of the rumen microorganisms and blond plasma protein in sheep. Acta Physiol. Pol. 33, 23-28

Kowalczyk J., Voigt J., Michalski J.P., Czauderna M., 2010. Efficiency of endogenous urea nitrogen incorporation into the ruminal bacteria and milk protein in goats fed diet differentiated in protein level. In: Proceedings of 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition. Parma (Italy). EAAP Publication No. 127, pp. 141-142

Lahann P., Voigt J., Kühn C., Pfuhl R., Metges C.C., Junghans P., Schönhusen U., Hammon H.M., 2010. Energy expenditure, urea kinetics, and body weight gain within a segregating resource family population. J. Dairy Sci. 93, 5118-5128

Lapierre H., Lobley G.E., 2001. Nitrogen recycling in the ruminant: A Review. J. Dairy Sci. 84, Suppl. E, E223-E236

Marini J.C., Van Amburgh M.E., 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81, 545-552

Meyer R.M., Bartley E.E., Deyoe C.W., Colenbrander V.F., 1967. Feed processing. I. Ration effects on rumen microbial protein synthesis and amino acid composition. J. Dairy Sci. 50, 1327-1332

Ndibualonyi B.B., Rodriguez M.N., Dehareng D., Cirio A., Godeau J.M., 1998. Effects of late pregnancy and early lactation on renal urea handling in Corridale ewes. J. Agr. Sci. 130, 213-216

Pfeffer E., Speckter H., Bornemann S., Holthausen A., Rodehutscord M., 2009. Kinetics of endogenous urea nitrogen in lactating goats and cows fed diets varying in their crude protein concentrations. Arch. Anim. Nutr. 63, 230-242

Rémond D., Meschy F., Boivin R., 1996. Metabolites, water and mineral exchanges across the rumen wall: Mechanisms and regulation. Ann. Zootech. 45, 97-119

Tebot I., Faix S., Szanyiova M., Cirio A., Leng L., 1998. Micropuncture study on urea movements in the kidney cortical tubules of low protein feed sheep. Vet. Res. 289, 99-105

Virtanen A.I., 1964. Versuche über Milchproduktion ohne Protein unter ausschließlicher Verwendung von Harnstoff und Ammoniumstickstoff. Nachr. Gies. Hochsch.-Gesell. 33, 73-85

Voigt J., Steger H., 1967. Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörpern in biologischem Material mit Hilfe eines modifizierten Mikrodiffusionsgefässes. Arch. Tierernähr. 17, 289-293

Wang C., Liu J.X., Yuan Z.P., Wu Y.M., Zhai S.W., Ye H.W., 2007. Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 90, 2960-2965

Zhai S.W., Liu J.X., Wu Y.M., Ye J., 2007. Predicting urinary nitrogen excretion by milk urea nitrogen in lactating Chinese Holstein cows. Anim. Sci. J. 78, 395-399