RELATIONSHIP BETWEEN THE CONTENT OF RUMEN DEGRADABLE PROTEIN IN DAIRY COW DIETS AND EMISSION OF EXCRETED AMMONIA NITROGEN

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Summary. The current study investigated the effect of concentration and efficiency of use of rumen degradable protein (RDP) on the nitrogen content of excreta and urea content of urine, as well as on ammonia emissions. Ammonia emission from excreta is mainly affected by the urea concentration in urine which, in turn, mainly results from protein degradation and the efficiency of use of protein in the rumen. The variable component in diets with equal crude protein content (13.2 ± 0.13 %) was the type of protein, which was either highly rumen degradable barley (B) or slowly rumen degradable maize (M). The urine dry matter contained a significantly higher concentration of nitrogen (P = 0.0015) and urea (P = 0.0012) when the barley diet was fed. In conditions of similar ambient temperature (°C) and humidity (%) the average ammonia emission from excreta also increased (P = 0.0001). The results reveal that, from the viewpoint of reducing emissions, in addition to the crude protein content, the amount, efficiency of use and precise rationing of rumen degradable protein play an important role when diets containing low levels of crude protein are fed.
Key words: dairy cattle, ammonia emission, rumen degradable protein, urine, urea.

RYŠYS TARP DIDŽIAJAME PRIESKRANDYJE VIRŠKINAMŲ BALTYMŲ SUDĖTIES IR IŠSKIRIAMO AMONIAKO AZOTO EMISIJOS

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Santrauka. Eksperimento metu tyrėme melžiamų karvių didžiajame prieskrandyje virškinamų baltymų koncentracijos ir jų vartojimo efektyvumo įtaką su šlapimu ir išmatomis išsiskiriančio azoto ir šlapimo šlapalo sudėčiai, taip pat amoniako emisijai. Amoniako emisijai didžiausią įtaką daro šlapalo kiekis šlapime, kuris iš esmės priklauso nuo baltymų skilimo bei jų suvartojimo efektyvumo didžiajame prieskrandyje. Kintantis raciono komponentas su vienodu žalių proteinų kiekiu (13,2±0,13 proc.) buvo arba greitai didžiajame prieskrandyje virškinami miežiai (B), arba lėtai virškinami kukurūzai (M). Nustatėme, jog šlapimo sausosiose medžiagose azoto (p = 0,0015) ir šlapalo (p = 0,0012) koncentracija buvo ženkliai didesnė šeriant racionu, papildytu miežiais. Taip pat nustatėme, kad esant panašiai aplinkos temperatūrai (°C) ir drėgmei (proc.) padidėjo ir vidutinė amoniako emisija (p = 0,0001). Tyrimų rezultatai rodo, jog, sumažėjus emisijai ir padidėjus žalių proteinų kiekiui, didžiajame prieskrandyje virškinamų baltymų kiekis, jų vartojimo efektyvumas ir tikslus normavimas atlieka svarbų vaidmenį šeriant karves pašarais, kuriuose žalių proteinų yra mažai.
Raktažodžiai: melžiamos karvės, amoniako emisija, didžiajame prieskrandyje virškinami baltymai, šlapimas, šlapalas.

Introduction. One of the most environmentally hazardous gases emitted into the atmosphere from animal production is ammonia, which is the product of aerobic fermentation of excreta. According to the data of Galloway et al. (2004), human activities have resulted in the emission of 47 million tonnes of ammonia per year, of which livestock and poultry production account for about 68%. One of the priorities of the European Union's agricultural policy (91/676/EEC) is the reduction of emissions, including those of ammonia, from animal production. The nitrogen content and ammonia emissions from farm animal excreta are affected by numerous different factors. Excreted faecal protein contains microbial, endogenous and indigestible dietary protein. Endogenous and indigestible dietary protein consists mainly of amino acids or their fragments; 15 to 20% of microbial nitrogen is a component of nucleic acids. Nitrogen in the urine of cattle, sheep and pigs is mainly excreted as urea, and in smaller amounts as allantoin, hippuric acid and creatine. Due to the activity of ureolytic microorganisms in faeces, the rapid breakdown of urine urea begins after it has been excreted from the animal and has become mixed with faeces (Tamminga and Verstegen, 1996).

The main factors affecting ammonia emission are the amount and urea content of the excreted urine. The most important factor affecting the nitrogen content of excreta is the concentration and quality of protein in the diet and the efficiency of use of dietary protein by the animal. In the digestive tract of ruminants, dietary protein is microbially processed in the rumen. As a result, a large proportion is converted into microbial protein. The efficiency of microbial synthesis depends on the solubility and degradability of dietary protein, the presence and availability of energy (rumen fermentable carbohydrates) needed to promote synthesis, and on synchronizing the above mentioned processes in the rumen. Waste products of protein degradation − mainly ammonia − are absorbed through the ruminal epithelial cells into the blood, converted into nontoxic urea in the liver, and excreted with the urine (Tamminga and Verstegen, 1996; Hof et al., 1997). A portion of urea is recycled from the rumen into saliva. Kennedy and Milligan (1980) and Giraldez (1997) have reported that, if the ammonia concentration in the rumen decreases then the proportion of urea recycled into the saliva increases. The urea content of urine and ammonia emissions from excreta can be reduced by lowering the protein content of the diet, with minimal negative effects on production parameters. Frank et al. (2002) have compared the effect of diets containing different crude protein levels (140 *vs.* 190 g/kg DM) on ammonia volatilization, and have shown that with low protein content in the diet ammonia emissions are reduced by nearly 70%. Similar trends have been observed in the studies carried out by Swensson (2003): reducing the protein content of the diet from 190 to 130 g/kg DM reduced ammonia emissions from excreta by nearly 3 times. Nitrogen losses from excreta can also be reduced by regulating the amounts of rumen soluble, degradable and undegradable protein, in addition to lowering the overall protein content in the diet. Ammonia emission from excreta can be reduced mainly by lowering the content of rumen degradable protein (van Duinkerken et al., 2005). Reynal and Broderick (2005) have studied the effect of diets with different concentrations of rumen degradable protein on production parameters and nitrogen metabolism in dairy cows, and have reported that reducing the crude protein concentration from 188 to 177 g/kg on a DM basis and lowering the content of rumen degradable protein in the diet from 132 to 117 g/kg DM decreases urea concentration in the urine by 20%.

The objective of the current experiment was to explain the effect of diets with low crude protein levels, but with different degradable protein content to the ammonia emission from excreta, which was studied under laboratory conditions.

Materials and methods

Experimental plan

The cross-over experiment was conducted with four Estonian Holstein Friesian (EHF) dairy cows in their first lactation. The experiment was divided into three periods and lasted for 14 days, which comprised an 11-day preliminary period and a 3-day experimental period. In the first and third periods of the trial the cows' concentrate ration was barley-based, whilst in the second it was maize-based. The cows were separately fed two diets with a similar content of crude protein (CP) and metabolizable energy (ME). The diets differed in the content of rumen degradable protein (RDP) and the protein balance in the rumen (PBV).

Diets

The cows were housed in tie stalls and fed individually (restricted) on the basis of live weight and milk production two times (06.00 AM and 18.00 PM) per day. The variable components of the diets were barley or maize; other components (grass silage, sunflower extracted meal, minerals) were the same. The chemical composition of the feeds is given in Table 1. The diets were compiled assuming that RDP was a limiting factor for ruminal protein synthesis – the PBV was negative (Table 2). The ME content of the diets was calculated using the method of Oll (1995). The content of metabolizable protein, RDP and total digestible carbohydrates (TDC) as well as the PBV (Madsen, 1985), were calculated on the basis of equations used in the Finland (Tuori et al., 1996), modified to suit Estonian conditions, as described by Kärt et al. (2002).

Table 1. **Chemical composition and calculated nutritive values of feeds**

Parameter	Grass silage n = 6 Mean ± S.E.	Sunflower extracted meal n = 6 Mean ± S.E.	Barley n = 6 Mean ± S.E.	Maize n = 6 Mean ± S.E.
DM, g/kg feed	335 ± 10.7	891 ± 1.4	876 ± 0.8	879 ± 1.9
g/ kg DM:				
Crude protein	134 ± 4.6	360 ± 1.7	108 ± 1.4	83 ± 0.5
Ash	87 ± 4.1	76 ± 0.4	24 ± 0.5	13 ± 0.2
Crude fibre	280 ± 4.3	230 ± 5.7	66 ± 2.4	33 ± 1.0
MP	77 ± 0.3	145 ± 1.4	100 ± 0.1	118 ± 0.2
RDP	108 ± 3.7	234 ± 1.1	82 ± 4.2	42 ± 0.2
PBV	3 ± 4.0	139 ± 1.5	-57 ± 1.5	-108 ± 0.2
TDC	467 ± 5.4	269 ± 2.2	715 ± 3.1	792 ± 0.4
ME, MJ/kg DM	9.0 ± 0.03	11.0 ± 0.22	13.0 ± 0.02	14.0 ± 0.00

Feeds and intake

Feed samples were collected on the 12th and 14th day of each period. The nutrient content of the feeds (Table 1) was determined according to generally accepted methods (AOAC, 2005). Crude protein (CP) content was determined according to the Kjeldec Auto 1030 analyzer (N x 6.25), crude fibre (CF) content – by means of Foss Tecator Analyzer, ether extract content – by Soxtec System 1040 and ash content – by combustion at 550°C. The dietary intake of the experimental cows was recorded daily.

Table 2. **Diets in different periods**

Feeds	Periods	
	1 and 3 (Barley)	2 (Maize)
Grass silage, kg DM	11.7	11.7
Sunflower extracted meal, kg DM	0.5-0.7	0.3-1.7
Minerals, kg	0.1	0.1
Barley, kg DM	4.5-7.5	-
Maize, kg DM	-	4.7-6.1
CP level, g/kg DM	130 ± 0.15	136 ± 0.21
ME, MJ/kg DM	10.3 ± 0.04	10.6 ± 0.05
MP, g/MJ ME	8.3 ± 0.01	8.8 ± 0.04
RDP, g/MJ ME	9.9 ± 0.12	9.3 ± 0.12
PBV, g /kg DM	-274 ± 25.4	- 339 ± 19.9

Milk and live weight

The average milk production of the dairy cows is given in Table 3. Milk production and live weight of the cows were recorded at the beginning and at the end of each period (6 times). During the experiment the cows' average live weight was 605 ± 8 kg. Changes recorded in milk production and body weight were insignificant, probably because of the short duration of the experimental period.

Table 3. **Average milk production and percentage composition of milk in different periods**

Groups	Milk, kg Mean ± S.E.	ECM, kg Mean ± S.E.	Fat, % Mean ± S.E.	Protein, % Mean ± S.E.	Urea, mg/l Mean ± S.E.
Barley	25.0 ± 0.77	24.1 ± 0.81	3.80 ± 0.101	3.24 ± 0.060	377 ± 25.5
Maize	25.1 ± 1.03	23.8 ± 1.06	3.74 ± 0.086	3.15 ± 0.091	189 ± 10.4

ECM has been calculated using the equation of Sjaunja et al. (1990)

Excreta and ammonia emission

Urine and faecal samples were collected on the 12th and 14th days of the experimental period. Spot sampling began three hours after the morning feed. One urine and one faeces sample was taken from each cow. Faecal contamination of urine samples, and vice versa, was avoided as far as possible. For determining the level of ammonia emission, faecal and urine samples from the experimental cows were collected into hermetically closed plastic containers and stored in a refrigerator at + 4ºC. The samples were analysed for the level of dry matter, total nitrogen and NH_4–N; the urea content was also determined in urine samples. Total nitrogen was determined by the Kjeldahl method using a Kjeltec Auto 1030 Analyser; ammonia nitrogen was detected using a Kjeltec 2300 Analyzer Unit, Foss Tecator TM Technology (AOAC, 2005). The urea concentration in urine was determined by the urease-Berthelot reaction using a Kit obtained from Human Gesellschaft für Biochemia und Diagnostica mbH. Ammonia emission was determined under laboratory conditions with an ammonia measuring device constructed at our institute (Figure 1).

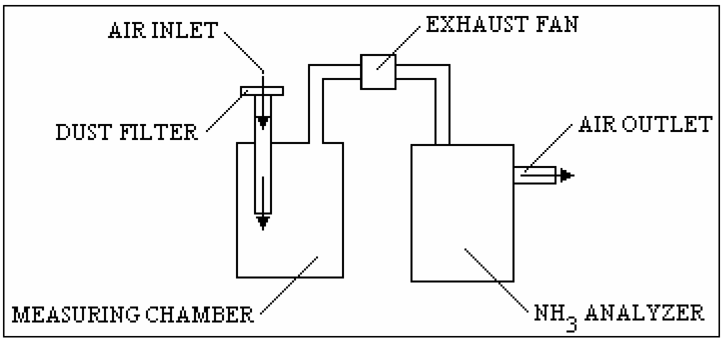

Figure 1. **Device for measuring ammonia**

5 g of urine and 5 g of faeces was mixed and placed in the measuring chamber for determining ammonia emission. Measurement of ammonia emission was begun one hour after preparing the sample. The area of the base of the measuring chamber was 14 cm^2, and this was kept open for ammonia volatilization. The volume of air flow from the measuring chamber was 0.15 m^3/h. The ammonia concentration in the air was measured using an elec-

trochemical unit Dräger PAC III (Draeger Safety Inc) with an interval of 1 min over a period of 18 h (by which time the batteries supporting the units were exhausted). To calculate the ammonia volatilized per hour, the following equation was used:

$E_{mg/h} = C \cdot q$, where:

E, amount of volatilized ammonia (mg/h);

C, ammonia concentration in exhausted air (mg/m^3);

q, air flow volume in the measuring chamber (m^3/h).

Statistical analysis

Statistical variation between the rations with respect to intake, and urine and faeces parameters, was analysed by repeated measures ANOVA using the MIXED procedure of SAS (SAS Institute, 2004) and the following model:

$Y_{ijk} = \mu + R_i + b{*}P_{ijk} + C_j + e_{ijk}$; where:

Y_{ijk}, investigated parameter;

μ, overall mean;

R_i, ration (i = 1 to 2);

b, corresponding regression coefficient;

P_{ijk}, period as continuous factor;

Cj, cow (j = 1 to 4);

e_{ijk}, residual error.

Results

Intake

No significant differences were found between the daily intake of dry matter (DM), metabolizable energy (ME), crude protein (CP) and total digestible carbohydrates (TDC) in the different experimental groups (Table 4). The composition of the diet in the experimental groups had a significant effect on the intake of metabolizable protein (MP) and rumen degradable protein (RDP): P = 0.0055 and P = 0.0265, respectively. The average daily consumption of metabolizable protein was 1574 g for the barley diet and 1667 g for the maize diet; the content of RDP was higher by 108 g when the barley diet was fed.

Table 4. **Total daily consumption of nutrients in different periods**

Parameter	Groups		P-value
	Barley n = 8+8 Least square means ± S.E.	Maize n = 8 Least square means ± S.E.	
DM, kg	18.3 ± 0.58	17.9 ± 0.62	0.3223
CP, kg	2,38 ± 0.09	2,44 ± 0.10	0.2701
ME MJ	189.3 ± 7.30	189.8 ± 7.59	0.8754
MP, kg	1,57 ± 0,07	1,67 ± 0.07	0.0055
RDP, kg	1,87 ± 0,07	1,76 ± 0.07	0.0265
PBV, g	-274 ± 22.1	-339 ± 31.3	0.1042
TDC, kg	9.95 ± 0.37	9,84 ± 0.38	0.6050

Faeces

The average chemical composition of faeces in different experimental groups is presented in Table 5. The diet composition and intake of nutritional factors had no effect on faecal dry matter content (P = 0.0608). A significant difference was revealed in the nitrogen content of faecal dry matter (P = 0.0138) and the NH_4-N content in total N (P = 0.001) in different groups.

Table 5. **Chemical composition of faeces in different periods**

Parameter	Groups		P-value
	Barley n = 8+8 Least square means ± S.E.	Maize n = 8 Least square means ± S.E.	
DM, g/kg faeces	145 ± 2.9	152 ± 3.4	0.0608
Total N g/kg DM	30 ± 0.4	32 ± 0.5	0.0138
NH_4-N, g/kg N	16 ± 1.1	6 ± 1.6	0.0001

Urine

The average parameters of the chemical composition of urine in the different experimental groups are given in Table 6. Dietary composition and intake of nutrients did not affect the urine dry matter content (P = 0.3607) or NH_4-N content in total N (P = 0.5737). A significant difference was observed between gruops in the concentration of total N and urea in urine dry matter: P = 0.0015 and P = 0.0012, respectively.

Temperature and relative humidity of air

Simultaneously with the measurement of emission parameters, the ambient temperature and relative humidity were recorded using a Testostor 175 data logger (Testo GmbH & Co). These parameters were also recorded with an interval of 1 min over a period of 18 h.

Ambient temperature is one of the main factors affecting ammonia emissions. The temperature ranged from 21.4–24.8 C (Barley) and 22.9–25.3 °C (Maize), and the relative humidity from 23.0–42.2 (Barley) and 24.3–32.1% (Maize). The difference between ambient temperature and relative humidity values in different measuring periods was minimal (P = 0.2359 and P = 0.5433). Thus it can be concluded that physical parameters of the environment had no significant effect on ammonia emissions in this experiment.

Ammonia emission

Table 7 presents the dynamics and average ammonia emissions from faeces+urine excreted by cows fed different diets. Ammonia emissions varied considerably. For the barley diet the variation was especially high and the average ammonia emission was 1.16–5.16 mg/h; for the maize diet the variability was somewhat lower: 1.27–3.52 mg/h.

Table 6. **Chemical composition of urine in different periods**

Parameter	Groups		P-value
	Barley n = 8+8 Least square means ± S.E.	Maize n = 8 Least square means ± S.E.	
DM, g/kg urine	65 ± 5.0	62 ± 5.3	0.3607
Total N, g/kg DM	93 ± 2.8	75 ± 3.9	0.0015
NH_4-N, g/kg N	3 ± 0.2	2 ± 0.3	0.5737
Urea, g/kg DM	81 ± 6.2	54 ± 7.4	0.0012

Table 7. **Dynamics of ammonia emission (mg/h) in different measuring periods**

Hours	Groups		P-value
	Barley n = 16 Least square means ± S.E.	Maize n = 8 Least square means ± S.E.	
1	1.47 ± 0.214	1.15 ± 0.303	0.4109
2	2.16 ± 0.290	2.12 ± 0.410	0.9354
3	2.60 ± 0.315	2.45 ± 0.446	0.7856
4	2.89 ± 0.319	2.59 ± 0.450	0.5849
5	3.14 ± 0.317	2.58 ± 0.448	0.3274
6	3.24 ± 0.306	2.51 ± 0.433	0.1859
7	3.31 ± 0.301	2.47 ± 0.426	0.1272
8	3.33 ± 0.300	2.41 ± 0.419	0.0899
9	3.46 ± 0.317	2.33 ± 0.448	0.0524
10	3.52 ± 0.309	2.22 ± 0.437	0.0261
11	3.58 ± 0.318	2.20 ± 0.449	0.0219
12	3.61 ± 0.315	2.14 ± 0.446	0.0145
13	3.71 ± 0.322	2.08 ± 0.455	0.0092
14	3.73 ± 0.307	1.99 ± 0.434	0.0042
15	3.70 ± 0.303	1.95 ± 0.429	0.0036
16	3.67 ± 0.290	1.93 ± 0.411	0.0028
17	3.75 ± 0.311	1.93 ± 0.432	0.0025
18	3.68 ± 0.326	1.91 ± 0.433	0.0021
Mean	3.25 ± 0.162	2.16 ± 0.177	0.0001

The amount of volatilized ammonia and the variation within the experiment was mainly affected by the urea concentration in the urine. The urea concentration also fluctuated over a wide range. The urea concentration varied from 54 to 109 g/kg DM urine for cows on the barley ration and from 27 to 76 g/kg DM urine for cows on the maize ration.

Discussion and conclusions

Several literature sources have reported that the nitrogen content of excreta and ammonia emissions from excreta are affected by the crude protein content of the diet (Frank et al., 2002; Swensson, 2003). On the basis of the experimental results presented here it can be claimed that the nitrogen content of excreta, especially that of urine, and ammonia emissions are affected by the crude protein concentration of the diet as well as by the percentage of the rumen degradable fraction in the protein. The degradation rate of RDP and the efficiency of use of ammonia by ruminal microbes are also of crucial importance (Vestergaard Thomsen, 1985). No statistically significant differences were found between the average intake of crude protein of the diets (P = 0.2701) and ME (P = 0.8754) in the different experimental groups. The difference between intakes of degradable protein in the experimental diets was related to the different ruminal degradability of the feeds (barley *vs.* maize). The ruminal degradability of barley protein is about 80% and the degradation is rapid. In contrast, the average ruminal degradability of maize protein is only 50% and the degradation is significantly slower (Madsen and Hvelplund, 1985).

Carbohydrates serve as the main source of energy for the synthesis of species-specific proteins by ruminal microbes. Different concentration and structure of carbohydrates in the experimental diets was also produced by the different feeds. Starch is the main non-structural carbohydrate in the feed, and is used by ruminal microbes as an energy source. The chemical composition of barley and maize starch is essentially different. The main component of barley starch is highly and rapidly rumen degradable amylose. Maize starch, on the other hand, consists mainly of ruminally undegradable amylopectin, which has no importance as an energy source for ruminal microbes. De Visser (1996), reviewing the literature on carbohydrates for dairy cows, concluded that when a barley-based diet is fed, the amount of microbial protein in the rumen is higher than when a maize diet is fed. In animals fed a barley diet, the ammonia concentration needed for ruminal protein synthesis is higher; a large amount of energy in the form of rumen degradable starch is also needed to promote the process. Based on the differences between the concentrations of urine nitrogen (9.3 *vs.* 7.5% DM, P = 0.0015) and especially between urea levels in the urine (81.0 vs. 54.4 g/kg DM, P = 0.0012) in the different experimental groups, it can be concluded that degradation of barley protein is so rapid and intensive that the amount of energy in the rumen (degradable starch) at that moment is not sufficient to bind the total amount of ammonia generated. This may explain the higher nitrogen and urea content in the urine excreted when a barley diet is fed.

The average faecal nitrogen content was higher in animals fed the maize diet (3.0 *vs.* 3.2% DM, P = 0.0138). This can be attributed to the higher content of crude protein as well as the lower content of RDP in the diet. A significant difference was found between the levels of ammonia nitrogen in faecal protein in the different groups. The content of NH_4-N in faeces was three times higher in animals on the barley diet (1.6 vs. 0.6%, P = 0.0001). This may have been due to the fact that some of the ammonia formed during protein degradation in the rumen is carried with the feed mass to the intestine and excreted in the faeces (Tamminga and Verstegen, 1996). Under similar environmental conditions, ammonia emission from excreta was 1.5 times higher (3.25 vs. 2.16 mg/h, P = 0.0001) when the barley diet was fed. This can be explained by the above-mentioned differences in protein structure of the experimental diets, especially by the concentration of RDP and the different efficiency of use of protein degradation products in the rumen. For the barley diet the nitrogen content in urine was 20% higher (9.2 vs. 7.5% DM, P = 0.0015) and urea content respectively 81.0 vs. 54.4 g/kg DM (P = 0.0012).

The dynamics of ammonia emission over time showed that emission was most intensive in the first half of the measurement period. No significant differences between experimental groups were observed (4 h, 2.89 *vs.* 2.59 mg/h, P = 0.5849). Beginning from the 4th hour of measurement, a considerable difference in emission between the groups was observed. Faecal ammonia emission increased in cows fed the barley diet and started to reduce when the maize diet was fed. In both experimental groups the emission of ammonia had stabilized by the 14^{th} measurement hour (3.73 vs. 1.99 mg/h, P = 0.0042). The differences in ammonia emission on the maize and barley diets can be explained mainly by the different nitrogen and urea content in the urine samples. Mixing of urine with faeces brings about rapid degradation of urine urea by the activity of ureolytic bacteria found in faeces, and its volatilization as ammonia. In contrast, microbial degradation of faecal protein, which consists mainly of amino acids and their fragments, is relatively slow (Tamminga and Verstegen, 1996).

The results of the present study reveal that, in addition to the low crude protein content of the diet, the level of ammonia emission from dairy cows is related to the amount of RDP.

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