EFFECT OF GRAIN SPECIES ON PURINE DERIVATIVE EXCRETION VIA URINE IN FEEDING LEGUMINOUS SILAGE TO RAMS

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Abstract. Alfalfa and clover silage protein is highly degradable in the rumen. Previous experiments have indicated that micro-organisms did not use leguminous protein effectively for protein synthesis. The aim of the present study was to find out, feeding on alfalfa and clover silage, the best grain species to increase purine derivative excretion via urine and thereby maximize the ruminal microbial protein synthesis. For that purpose, two trials were carried out with rams on the principle of 4 x 4 Latin square design in special metabolic cages. In the first trial the rams were fed on alfalfa silage, and in the second trial – on clover silage. The crude protein content in dry matter of silages was 20.3 % and 19.7%, respectively. Considering energy requirement, the leguminous silage and concentrates were fed as follows: 1) silage + barley (50:50), 2) silage + barley + oats (50:25:25), 3) silage + barley + wheat (50:25:25) 4) silage + barley + maize (50:25:25). Animals were fed twice a day on a maintenance level. Purine derivatives were determined and microbial protein synthesis was calculated according to Chen and Gomes (1992). In the present trial conditions the grain species did not affect purine derivative excretion. However, a significantly higher allontoin content in rams urine was observed when clover silage with barley was fed, compared with the ration containing alfalfa silage and barley ($P < 0.05$). We can not confirm the statement that rams use clover silage protein more effectively for microbial protein synthesis than that of alfalfa silage.

Keywords: leguminous silage, purine derivative, microbial protein synthesis, sheep.

JAVŲ VEISLIŲ ĮTAKA PURINO JUNGINIŲ IŠSKYRIMUI SU ŠLAPIMU, ŠERIANT AVINUS ANKŠTINIŲ AUGALŲ SILOSU

Santrauka. Liucernos ir dobilų silose esantys proteinai yra gerai skaidomi didžiajame prieskrandyje. Ankstesnių tyrimų duomenys rodo, kad mikroorganizmai nepakankamai efektyviai panaudoja ankštiniuose esančius proteinus proteinų sitezei. Šio tyrimo tikslas buvo nustatyti geriausias grūdų rūšis, leidžiančias padidinti purino juginių pašalinimą su šlapimu, šeriant liucernos ir dobilų silosu, ir kartu padidinti mikrobinę proteinų sintezę prieskrandžiuose. Šiuo tikslu buvo atlikti du bandymai su avinais 4×4 lotynų kvadrato principu specialiuose garduose medžiagų apykaitai tirti. Pirmojo bandymo metu avinai buvo šeriami liucernos, antrojo – dobilų silosu. Žaliųjų proteinų kiekis siloso sausoje medžiagoje buvo atitinkamai 20,3 % ir 19,7 %. Atsižvelgiant į energijos poreikį, ankštinių silosas buvo šeriamas taip: 1) silosas + miežiai (50:50), 2) silosas + miežiai + avižos (50:25:25), 3) silosas + miežiai + kviečiai (50:25:25), 4) silosas + miežiai + kukurūzai (50:25:25). Gyvuliai buvo šeriami du kartus per dieną palaikymo lygiu. Purino junginiai buvo nustatomi ir mikrobinių proteinų sintezė apskaičiuojama Chen ir Gomes metodu (1992). Šiomis bandymo sąlygomis grūdų rūšis neturėjo įtakos purino junginių išskyrimui. Tačiau, nors šeriant avinus dobilų silosu su miežiais, šlapime buvo nustatytas žymiai didesnis alantoino kiekis, palyginti su racionu, sudarytu iš liucernos, siloso ir miežių ($P<0.05$), negalime teigti, kad avinai dobilų siloso proteinus panaudoja mikrobinių proteinų sintezei efektyviau negu liucernos siloso.

Raktažodžiai: ankštinių silosas, purino junginiai, mikrobinė proteinų sintezė, avys.

Introduction. It is established that silage protein is highly degradable in the rumen (McDonald et al., 1991). It has also been ascertained that protein degradability of leguminous silage in the rumen is significantly higher than that of gramineous silage (Fraser et al., 2000; Jones et al., 1995; Albrecht & Muck, 1991). Such highly degradable feed protein is not effectively used by ruminal microbes, as due to a lack of energy, a large amount of released ammonia is not used for microbial protein synthesis. The unused ammonia, absorbed into blood through rumen wall, cannot always be rapidly changed to urea in the liver. Therefore, animals may suffer from metabolisable diseases (Strang et al., 1998) and fertility problems (Butler, 1998; Webb et al., 1999). The urea content in blood and milk, and the quantity of unused protein will increase both in urine and manure (Shingfield, 2001), especially in the middle and at the end of lactation (Kalscheur et al., 1999). All this refers to an ineffective use of silage, (particular leguminous silage), by ruminal microbes.

The effectiveness of microbial protein synthesis in the rumen is directly related to a simultaneous and synchronous release of energy attainable by micro-organisms both in degradable protein and carbohydrates fermentation. Feed carbohydrates, depending on their physical and chemical characteristics, are hydrolyzing the ruminal micro-organisms with different speed and scope (Stern et al., 1994).

To improve utilization of ruminal ammonia with silage diets, sugar supplements have been suggested (van Vuuren et al., 1990). Khalili and Huhtanen (1991) showed that sucrose supplementation was effective in decreasing

ruminal ammonia nitrogen and increasing microbial protein synthesis, but continuos infusion of sucrose stimulated microbial protein synthesis more than twice daily feeding, despite the latter providing a more synchronous supply of energy and nitrogen. Also, it has well been proved that in the case the content of non-structural carbohydrates in the ration increases, the content of microbial protein in the rumen increases as well (Russell et al., 1992).

In recent years the growing area of legumes and their utilization in dairy cows rations has been increased in Estonia. Potatoes and several root crops are not used in the rations any more. To balance the rations, more and more different grain species and oil-processing by-products are being used. As leguminous protein is ruminally rapidly degradable, and starch of different grains hydrolyzes at different rate in the rumen (Sauvant & Milgen, 1995), we set up the goal of our experiments to explain in which case of grain species or their mixtures in the ration the utilization of alfalfa or clover protein by ruminal micro-organisms is most effective.

The determination of the amount of ruminal protein synthesis is essential in contemporary protein evaluation systems, and urinary excretion of purine derivative (PD) appears to be a reliable non-invasive method to estimate microbial protein flow to the duodenum in ruminants. The amount of PD excreted via urine is quite well correlated with the amount of ruminally synthesized microbial protein (Puchala & Kulasek, 1992; Lindberg et al., 1989).

Materials and Methods. Two trials were carried out with rams on the principle of 4 x 4 Latin square design in special metabolic cages. In the first trial the rams were fed on alfalfa silage, and in the second trial – on clover silage. The preliminary period lasted for 11 days; the duration of the trial period – 7 days. The rams were weighed prior to each trial period.

Considering the energy requirement (8 MJ metabolizable energy (ME) and 60 g crude protein (CP) for 50 kg ram per day (Futterwerttabellen, 1997), in both trials the animals were fed leguminous silage and concentrate twice a day on a maintenance level (Table 1) as follows: 1) silage + barley (50:50), 2) silage + barley + oats (50:25:25), 3) silage + barley + wheat (50:25:25), and 4) silage + barley + maize (50:25:25).

In the trial period, feeds and feed residues were sampled and dried at 60ºC, and milled. Dry matter (DM) was determined by drying at 105ºC to constant weight. 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 550ºC. Cell wall components were determined with detergent solutions according to the method by van Soest (1994).

Table 1. **Chemical composition of feeds used in the trials**

Items	Alfalfa silage	Clover silage	Barley	Oats	Wheat	Maize
Dry matter %	24.19	23.74	83.67	85.24	83.72	84.88
Composition of DM						
Crude protein %	19.87	19.68	12.44	13.10	15.73	10.93
Ash %	9.77	9.44	2.37	2.74	2.14	1.41
Crude fibre %	31.23	19.01	5.27	11.32	3.40	2.94
Neutral-detergent fibre (NDF) %	48.70	33.31	21.21	31.30	14.58	12.76
Acid-detergent fibre (ADF) %	34.76	23.38	5.91	13.55	3.63	3.26
Ether extract %	4.61	6.22	1.72	4.41	2.17	3.95
Non-fibre carbohydrates (NFC) %	34.52	45.65	78.20	68.44	76.57	80.77
Metabolizable energy MJ/kg	9.29	9.73	12.50	11.05	13.66	13.64
Metabolizable protein (MP) g/kg	75.1	78.0	103.8	90.2	107.3	126.0
Rumen protein balance g/kg	69.0	62.6	-45.9	-15.0	-19.2	-93.1
Organic matter (OM) %	90.2	90.6	97.6	97.3	97.9	98.6
Organic matter digestibility %	62.25	64.00	83.00	69.00	88.00	89.88

In the trial period urine was collected, weighed and sampled once a day for further analysis. The urine samples were processed, preserved and analyzed for allantoin, xanthine and hypoxanthine content according to Chen and Gomes procedure (1992). Uric acid was determined by using enzymatic colorimetric test (PAP – method with Lipid Clearing Factor). The sum of allantoin, uric acid, xanthine + hypoxanthine is referred to as total PD. Urinary urea was measured by enzymatic UV kinetic test (Human Gesellschaft für Biochemica und Diagnostica mbH) and total urinary nitrogen content - by Kjeldec Auto 1030 Analyzer. The synthesis of microbial nitrogen in the rumen was calculated on the basis of total PD excretion via urine according to Chen and Comes (1992). Results were analysed using computer program Microsoft Excel 97.

Results. In both trials the rams were fed on energy basis, according to maintenance level (Table 2). However, due to high CP content, the rams got daily about 55 to 65 g CP more than needed for maintenance. Despite the concentrates of different composition, fed to rams in addition to alfalfa and clover silage, there were no statistically significant differences between DM, OM, CP, MP and ME intake. Neither did grain species affect NFC intake. However, the markedly higher NFC intake was observed in feeding on clover silage, compared with

alfalfa silage. This was caused by higher NFC content of clover silage. As a comparison between the two trials indicated, the cell wall matter intake was significantly higher in feeding with alfalfa silage, which was related to its higher CF, NDF and ADF content. Moreover, in both trials certain significant differences were found in cell wall matter intake, related to higher CF, NDF and ADF content of oats, compared with other grain species used in the trial.

Table 2. **Main results of the trials**

Items	Alfalfa silage				Clover silage			
	Barley	Barley + oats	Barley + wheat	Barley + maize	Barley	Barley + oats	Barley + wheat	Barley + maize
	a	b	c	d	e	f	g	h
Nutritive factors intake per day								
Dry matter g	721.5 ± 19.0	738.1 ± 29.6	714.6 ± 9.4	692.2 ± 58.2	735.5 ± 39.5	752.8 ± 39.2	720.4 ± 38.1	725.4 ± 41.1
Organic matter g	673.2 ± 17.1	689.0 ± 27.0	666.5 ± 8.5	645.9 ± 56.7	688.0 ± 36.2	704.4 ± 36.0	673.7 ± 34.7	680.3 ± 38.5
Crude protein g	121.1 ± 1.7^{c}	123.8 ± 4.0	124.9 ± 1.5^{a}	115.4 ± 8.4	121.3 ± 6.6	124.8 ± 6.5	124.1 ± 7.1	117.9 ± 7.0
Metabolizable protein g	62.8 ± 1.1	62.2 ± 2.0	62.5 ± 0.7	62.6 ± 6.7	65.3 ± 3.0	64.9 ± 3.0	64.3 ± 2.8	67.6 ± 3.1
Metabolizable energy MJ	7.7 ± 0.2	7.6 ± 0.3	7.7 ± 0.1	7.4 ± 0.7	8.0 ± 0.4	8.0 ± 0.4	8.0 ± 0.4	8.1 ± 0.4
Crude fibre g	146.7 ± 13.5^{e}	157.8 ± 15.0^{f}	145.0 ± 10.3^{g}	142.4 ± 9.5^{h}	99.1 ± 8.8^{a}	109.8 ± 8.6^{bh}	95.0 ± 8.7^{c}	94.6 ± 8.9^{df}
Neutral-detergent fibre g	266.3 ± 13.3^{e}	286.7 ± 16.9^{cdf}	257.5 ± 8.1^{bg}	251.7 ± 11.8^{bh}	211.5 ± 19.7^{a}	232.2 ± 19.6^{bgh}	197.8 ± 19.1^{cf}	194.1 ± 19.9^{df}
Acid-detergent fibre g	164.6 ± 11.1^{e}	177.9 ± 13.0^{df}	162.0 ± 7.5^{g}	159.0 ± 7.3^{bh}	117.8 ± 10.3^{a}	131.8 ± 10.2^{bgh}	113.5 ± 10.2^{cf}	113.4 ± 10.4^{df}
Non-fibre carbohydrates g	380.5± 4.1^{e}	377.3± 8.7^{f}	371.2± 8.2^{g}	361.0± 48.8^{h}	436.8± 25.6^{a}	434.4± 26.2^{b}	423.2± 22.8^{c}	433.0± 26.0^{d}
Urinary-N, urinary urea-N and PD excretion per day								
Urinary-N g	9.3 ±1.2	9.4 ±1.5	10.0 ±1.6	9.5 ± 1.4	9.3 ± 0.9	9.6 ± 1.3	9.4 ± 1.2	8.5 ± 0.7
Urinary urea-N g	7.5 ± 1.1	9.0±2.0	8.7 ± 1.5	7.3 ± 1.6	6.1 ± 0.4	7.3 ± 1.3	7.2 ± 2.1	6.8 ± 2.5
Allantoin mg	812 ± 112^{e}	836 ± 217	866 ± 103	782 ± 130	1014± 108^{a}	965 ± 75	907 ± 132	885 ± 65
Uric acid mg	68 ± 7	66 ± 13	73 ± 20	73 ± 24	88 ±23	85 ± 15	80 ± 16	86 ± 33
Xanthine + hypoxanthine mg	92 ± 12	79 ± 16	90 ± 7	86 ± 11	85 ±21	75 ± 39	81 ± 35	73 ± 11
Total PD mg	973 ± 117	981 ± 232	1029 ± 111	941 ± 149	1187± 147	1125± 95	1068± 158	1044± 70
Computed microbial-N synthesis per day								
Microbial-N g	4.9 ±0.8	4.9 ±1.5	5.3 ± 0.7	4.7 ± 1.0	6.2 ±0.9	5.8 ± 0.6	5.4 ± 1.0	5.3 ± 0.4
a,b,c,d,e,f,g,h - $P < 0.05$								

The rams used in the trials excreted via urine from 8.5 to 10.0 g nitrogen and from 6.1 to 9.0 g urinary urea-N a day. The studied leguminous silages and species of grain did not affect statistically significantly the quantity of excreted nitrogen and urinary urea-N.

As for PD, the excretion of allantoin was most significant – 83.1 to 85.8 %. The share of uric acid and xanthine + hypoxanthine in the excreted PD constituted 6.7 to 8.2 % and 6.7 to 9.5 %, respectively. In case of feeding alfalfa silage to rams, the share of xanthine + hypoxanthine in the quantity of excreted PD was bigger than that of uric acid; in case of feeding clover silage the shares of xanthine + hypoxanthine and that of uric acid in the amount of excreted PD were approximately equal.

Some what higher excretion of PD was observed in case of feeding the experimental animals with clover silage, compared with alfalfa silage. However, the allantoin excretion was statistically plausible only in case when, besides silage, supplemental barley meal as the only grain species was fed to rams. The effect of grain

species on excretion of PD was statistically significant neither in case of feeding clover silage nor in feeding alfalfa silage.

A statistically significant correlation was observed between the daily amount of excreted allantoin and CF intake ($r = -0.4817$, $P<0.05$), ADF intake ($r = -0.4348$, $P<0.05$) and NFC intake ($r = 0.5290$, $P<0.005$); between uric acid excretion and CP intake ($r = 0.3594$, $P<0.05$) MP intake ($r = 0.4036$, $P<0.05$), ME intake ($r = 0.4094$, $P<0.05$) and NFC intake ($r = 0.3916$, $P<0.05$). There was no statistically significant correlation between xanthine + hypoxanthine intake and any of the determined nutritive factors.

The experimental animals synthesized from 4.7 to 6.2 g of microbial nitrogen a day (calculated according to excretion of PD). Although in case of feeding on clover silage more microbial protein was synthesized than in case of feeding on alfalfa silage, the difference was not statistically significant. Moreover, the microbial protein synthesis was not statistically significantly affected by grain species either.

The microbial nitrogen synthesis in the rumen is negatively affected by both CF intake ($r = -0.4244$, $P<0.05$) and ADF intake ($r = -0.3865$, $P<0.05$), and positively – by NFC intake ($r = 0.4772$, $P<0.01$). In these trials the amount of synthesized nitrogen correlated neither with DM, OM, CP, MP nor ME intake.

Discussion. In these trials the rams were fed on energy basis according to maintenance requirements. As the alfalfa and clover silages of extremely high protein content were used, the amount of ruminally degradable protein could not be the limiting factor of the microbial protein synthesis. It was presumed basing on the previous studies (Herrera-Saldana et al., 1990, Aldrich et al., 1993) that in supplementation of barley meal in the ration, a bigger amount of microbial protein could be synthesized in the rumen than in case of cornmeal supplementation. Our study results did not confirm that synchronization for rapid protein fermentation with the more degradable starch stimulated greater microbial protein passage, as reported by Herrera-Saldana et al. (1990). Our study results confirm the statements by Casper et al. (1999) and Henning et al. (1993) that improving synchronization of energy and nitrogen supply does not increase microbial protein synthesis and that efforts should be directed at manipulating ruminal energy supply. Further research is needed to study the effect of relationships between carbohydrate and protein sources on ruminal fermentation, microbial protein synthesis, and animal performance, especially when the source on carbohydrates is barley.

Although several researchers have found that the less extensive protein breakdown observed in clover silage, compared with alfalfa silage (Winters et al., 1999), and losses of nitrogen via urine are bigger in case of feeding the rams on alfalfa silage than in feeding on clover silage (Mariecia et al., 1999); our studies did not show similar results. However, the rams excreted statistically plausibly more allantoin in case of feeding on clover silage + barley meal than in case of feeding with alfalfa silage + barley meal ($P = 0.0404$), the difference between groups in microbial protein synthesis was not statistically plausible ($P = 0.0783$).

The reasons why the microbial protein synthesis was not statistically plausibly affected by DM, OM, ME, CP and MP intake can probably be found in the methods used in these trials. As the rams were fed on energy basis, according to maintenance level, and there were no statistically significant differences observed concerning DM, OM and ME intake, it did not affect the microbial protein synthesis in the rumen either. As CP and MP were not the limiting factors of microbial protein synthesis, they could not have any effect on the amount of synthesized protein.

Conclusions. In the present trials the grain species did not affect PD excretion and microbial protein synthesis in rams. However, a significantly higher allontoin content in rams urine was observed when clover silage with barley was fed, compared with the ration containing alfalfa silage and barley ($P<0.05$); we can not confirm the statement that rams use clover silage protein more effectively for microbial protein synthesis than that of alfalfa silage. Therefore, further research is needed to investigate kinetics of protein and carbohydrate degradability and microbial protein synthesis in the rumen.

Acknowledgements. The authors wish to thank Mrs Ülle Laurits for care and handling of the animals and Mrs Aime Pärn and other staff for the laboratory analysis. The research work was supported by the Estonian Science Foundation (Grant 3707).

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2002-09-26