THE EFFECT OF BARLEY MEAL ON DAIRY COW'S DRY MATTER INTAKE, ALLANTOIN EXCRETION AND MICROBIAL PROTEIN SYNTHESIS IN FEEDING ALFALFA SILAGE *AD LIBITUM*

O. Kärt, M. Ots, E. Rihma, H. Kaldmäe

KOKKUVÕTE: Odrajahu mõju lüpsilehmade kuivaine söömusele, allantoiini ekskretsioonile ja mikroobse proteiini sünteesile lutsernisilo* ad libitum *söötmise korral. *Katse korraldati vatsafistulitega varustatud holsteini tõugu lehmadega 4×4 ladina ruudu põhimõttel. Katse eesmärgiks oli selgitada odrajahu mõju lutsernisilo* ad libitum *söötmise korral silo söömusele, allantoiini ekskretsioonile ja mikroobse proteiini sünteesile. Lehmadele söödeti lisaks lutsernisilole odrajahu kas 25, 40, 55 või 70% nende metaboliseeruva energia tarbest. Katseloomi söödeti kaks korda päevas, hommikul kell 5.00 ning õhtul kell 17.00. Katse eelperiood kestis 8 päeva ja katse põhiperiood 6 päeva. Vatsavedeliku proovid võeti viiendal ja kuuendal katsepäeval, neli tundi pärast hommikust söötmist. Uriin koguti kuuendal katsepäeval 12 tunni jooksul põiekateetriga. Odrajahu osatähtsuse suurenemise korral ratsioonis 25%-lt kuni 70%-ni vähendas iga täiendavalt söödaratsiooni võetud odrajahu kilogramm silo kuivaine söömust 1,02 kg võrra. Uriiniga eritatud allantoiini kogus korreleerus statistiliselt usutavalt kuivaine (r=0,553; P<0,05), metaboliseeruva energia (r=0,665; P<0,001) ja kiuvabade süsivesikute – NFC (r=0,662; P<0,001) söömusega, vatsas seedunud orgaanilise ainega – DOMR (r=0,664; P<0,01), uriini üld-N sisaldusega (r=0,562; P<0,05) ning päevas eritatud üldlammastiku kogusega (r=0,0,601; P<0,01). Iga kilogrammi vatsas seedunud orgaanilise aine kohta sünteesiti mikroobset N vastavalt katsevariandile 24,7; 23,2; 28,3 või 29,8 g ja iga MJ metaboliseeruva energia kohta 1,03; 0,96; 1,14 või 1,22 g. Lutsernisilo* ad libitum *söötmise korral ei taga odrajahu lisasöötmine maksimaalset mikroobse proteiini sünteesi lüpsilehmade vatsas.*

Abstract

Four ruminally cannulated Holstein cows in midlactation were randomly assigned to a 4×4 Latin square design to evaluate barley meal effect on alfalfa silage dry matter intake, allantoin excretion and microbial protein synthesis. Cows were fed alfalfa silage (37.2% dry matter and in dry matter 21.9% crude protein, 35.4% NDF and 29.4% ADF*) ad libitum* and barley meal equivalent to 25, 40, 55 or 70% of their metabolic energy requirement. The preliminary period lasted for 8 and the trial period for 6 days. Urine was collected on the 6th day of the trial period during 12 hours with bladder catheters. Samples of rumen fluid were taken on the 5th and 6th day of the trial period 4 hours after morning feeding. Barley meal proportion in the ration was increased from 25% to 75%. Each additional kg of barley meal in the ration decreased the dry matter intake of alfalfa silage by 1.02 kg. Urinary allantoin excretion correlated with DM intake (r=0.553; P<0.05), ME intake (0.665; P<0.001) non-fibre carbohydrate (NFC) intake (r=0.662; P<0.001), digestible organic matter fermented in the rumen (DOMR) content in the ration (r=0.664; P<0.01), urinary N content (r=0.562; P<0.05) and daily N excretion (r=0.601; P<0.01). Respectively to the trial variant, the amount of synthesized ruminal microbial nitrogen per each kg of DOMR was 24.7; 23.2; 28.3 and 29.8 g and for MJ^{-1} metabolizable energy these values were 1.03; 0.96; 1.14 and 1.22 g. When alfalfa silage is fed *ad libitum*, barley meal as the only source of nonstructural carbohydrates in the ration of cows is not sufficient for synchronizing the hydrolysis of energy and protein and for maximizing microbial protein synthesis in the rumen.

Keywords: alfalfa, intake, purine derivatives, allantoin, microbial protein synthesis.

Introduction

The ruminants cover 60…100% of their protein requirement with ruminally synthesized microbial protein. As the amount of ruminal microbial protein does not cover the protein requirement of dairy cows it would be very important to know how much microbial protein is synthesized in the rumen in the case of every single feed or ration.

The *in vivo* methods of determining the quantity of ruminal microbial protein are based on the usage of various markers. The most widely used markers are the nucleic acids of micro-organisms (markers inside an organism) or marked atoms, eg. ^{15}N and/or ^{35}S (markers outside an organism). Such markers enable us to determine the quantity of ruminally synthesized microbial protein only in the case the trial cows are equipped with ruminal, duodenum or abomasums fistulas. Additionally it is always necessary to determine the rate of feed passage in the digestive tract. As all these methods are labour consuming and need animals to be specially

prepared for the investigation, the method based on the determination of purine derivatives (PD) have been elaborated in a recent decade. As this method is based on the determination of metabolic products of microbial protein, PD are also called metabolic markers.

The method of determining PD is based on the fact that the nucleic acids in the duodenum are mainly of microbial origin, digested in the digestive tract by enzyms (pancreatic ribonuclease and nuclease, nucleotidase etc.), absorbed in the small intestine as adenine and quanine, catabolize to final products which are excreted from the organism with urine. In ruminant's urine the amount of allantoins is the highest compared with other PD but also uric acid, xanthine and hypoxanthine can be found. Yet in cattle's urine only allantoin and uric acid can be found as the activity of xanthine oxidase in cattle's blood and tissues is high and xanthine and hypoxanthine are hydrolyzed into uric acid before excreted from the organism (Chen *et al*., 1990). Only in cases feeds contain much rumen-undegradable protein (fish meal, alfalfa meal) somewhat more nucleic acids originated from feeds enter the duodenum. Therefore the amount of true ruminally synthesized microbial protein can be overestimated in some cases when the method based on determining PD is used (Shingfield, 1996).

Determining the quantity of PD excreted by urine we have to take into account the fact that not 100 per cent of microbial nucleic acids is digested nor absorbed in the small intestine. Simultaneously a small amount of endogenous PD which originate from the catabolism of the body cells can be found in the urine. The digestibility of microbial nucleic acids, their absorption in the small intestine and the quantity of endogenous PD excreted with urine are sufficiently constant characteristics and they do not depend on feeds significantly. Verbic *et al*. (1990) have found that the digestibility of microbial nucleic acids is 83% and their absorption efficiency 85%. The quantity of endogenous PD excreted with urine depends mainly on the metabolic mass of an animal. The trials have proved that per kg^{-1} metabolic mass of the cattle 385 Mmoles endogenous PD are excreted with urine (Verbic *et al*., 1990).

As the ratio of allantoin and uric acid in the cattle's urine is quite constant it would be enough to determine only allantoin in the urine in order to estimate the quantity of ruminal microbial protein. Allantoin makes up 85% of the total PD excretion by the urine (Chen, Gomes, 1992).

The former trials have indicated that protein in grass (Goblentz *et al*., 1991; Cassida *et al*., 2000), especially in silage prepared from them (McDonald *et al*., 1991) is easily degradable in the rumen. The ruminal protein degradability is increased by very intensive proteolytic processes taking place during wilting and ensiling the grass (McDonald *et al*., 1991). Several trials (Fraser *et al*., 2000; Hatfield and Muck 1999; Jones *et al*., 1995; Albrecht and Muck, 1991) have indicated that in ensiling process especially alfalfa protein tends to hydrolyze to a large extent.

In recent years the growing area of alfalfa has increased in Estonia and on many farms it has become the main grass feed for the cattle in winter. Thus we set up the task to clarify in our trial how the amount of barley in the dairy cows ration affects alfalfa silage intake, fermentation processes in the rumen and the amount of protein synthesized in the rumen. In order to determine the latter we used the method based on PD.

Materials and Methods

Four lactating cows housed in individual stalls and fitted with ruminal fistulas were assigned to a 4×4 Latin square design. They were fed alfalfa silage, barley meal (Table 1) and mineral vitamin mixture. Cows were fed alfalfa silage *ad libitum* and barley meal equivalent to 25%, 40%, 55% or 70% of their metabolic energy requirement. The calculation of requirement of the cows was based on energy allowances compiled by Oll (1995). The cows were fed twice a day, at 5 a.m. and 5 p.m.

The preliminary period lasted for 8 and the trial period for 6 days. During the experiment the intake of alfalfa silage and barley meal was measured. Fodder and its residue were analyzed by the proximate analysis of feedstuffs, NDF and ADF by ANKOM equipment. Live masses of the cows were determined by a tape measure before the each trial period.

Urine was collected on the 6^{th} day of the trial during 12 hours from 7 a.m. to 7 p.m. with bladder catheters. To determine the amount of allantoin representative urine samples were collected. 10% H_2SO_4 was added to prevent PD from bacterial decomposition. To avoid sedimentation, samples were diluted with water and stored at 20º C. Chen, Gomes (1992) procedure was used to determine allantoin in the urine. Urinary urea was determined by urea-Berthelot' reaction and total urinary N content by Kjeltec Auto 1030 Analyzer.

Samples of rumen fluid were taken on the 5^{th} and 6^{th} days of the experiment at 9 a.m. by vacuum pump. The samples were squeezed through two layers of cheesecloth and pH was measured immediately by portable Sentron pH-System 1001 analyzer. Ammonia N and total acidity were measured by Kjeltec Auto 1030 Analyzer in the presence of MgO, VFA by gas-chromatography (gas-chromatograph Chrom-5).

DOMR was calculated on ARC (1984) basis, taking into account that 65% of the organic matter ferments in the rumen. The total PD excretion was computed on the basis of allantoin content, considering that allantoin makes up 85% of the total PD (Chen and Gomes, 1992). Absorbed microbial PD was found by subtracing the endogenous PD excretion from the total PD excretion and dividing it by 0.85 as 0.85 is PD absorptive efficiency (Valadares *et al*., 1999). The synthesis of microbial-N in the rumen was calculated as follows: 1. Through

estimated PD, according to Valadares *et al*. (1999) calculations; 2. On the basis of DOMR, considering that for each kg of DOMR, 32 g of ruminal microbial-N is synthesized (ARC 1984); 3. Through ME, considering that 1.4 g microbial-N is synthesized for MJ^{-1} ME (ARC 1984).

Table 1. Chemical composition and nutritive value of alfalfa silage and barley meal
Tabel 1. *Lutsernisilo ja odrajahu keemiline koostis ja toiteväärtus*

Item *Näitaja*	Alfalfa silage *Lutsernisilo*	Barley meal *Odrajahu*
Dry matter, % *Kuivaine, %*	37.17	88.45
In dry matter: *Kuivaines*		
Crude protein, % *Toorproteiin, %*	21.92	14.07
Crude ash, % *Toortuhk, %*	11.51	2.78
Crude fat,% *Toorrasv, %*	3.69	2.71
Nonfibre Carbohydrates, % *Kiuvabad süsivesikud*, %	37.83	74.13
Ca, g/kg	14.85	0.93
P, g/kg	3.40	2.32
Butyric acid, % *Võihape, %*	0.02	–
pH	4.90	–
ME, MJ/kg	9.03	12.69
NDF, %	35.39	27.95
ADF, %	29.44	7.56

Results and Discussion

The effect of barley meal on silage intake and milk composition

The accurate estimation of DMI of dairy cows is essential for ration formulation. When silage is fed *ad libitum* it is very important to know the factors affecting silage DMI. Factors used most frequently to predict *ad libitum* DMI were body weight (BW), fat-corrected milk (FCM) yield, milk protein yield, days in milk (DIM), ration crude fiber or NDF content, percentage of DM in silage, ambient temperature (Holter *et al*., 1997; Fuentes-Pila *et al*., 1996). In recent years more attention has been paid to studing the effect of concentrates, especially that of starch, on silage dry matter intake. De Visser *et al*. (1998) fed the cows silage prepared from perennial rye grass *Lolium perenne* harvested in the early or late maturity stage and various amounts of rumen–degradable starch in composition of total mixed ration (TMR). The authors found that when 4 kg flaked corn starch was added to TMR, the dry matter intake decreased by 15.1% when silage prepared from perennial rye grass in late maturity stage was used, and by 5.3% when silage prepared from grass of early maturity stage was used.

Our trial revealed that when the cows were fed alfalfa silage and different quantities of barley meal the negative effect of the latter on silage DMI was significant. In the case barley meal dry matter in the cows ration was increased from 3.4 to 8.1 kg per day, silage dry matter intake decreased from 13.8 to 9.0 kg per day (Table 2). Thus each kg of additional barley meal dry matter in the ration decreased alfalfa silage DMI by 1.02 kg.

NDF intake per 100 kg live weight ranged from 1.18 to 1.11 kg and this is in good correspondence with Mertens (1987) investigations . The increase of barley meal proportion in the ration decreased NDF intake but in that trial it was not statistically significant.

Although the increase of barley meal proportion in the ration increased ME intake and energetic density of the ration to some extent, no significant effect on cows milk production, nor on protein and urea content of milk was observed. High barley meal content in the ration, however, decreased the fat content of milk.

Table 2. The effect of barley meal on alfalfa silage intake and milk composition
Tabel 2. *Odrajahu mõju lutsernisilo söömusele ja piima koostisele*

Item *Näitaja*	Barley meal proportion of energy need, % *Odrajahu osatähtsus energiatarbest, %*			
	25 a	40 b	55 c	70 d
Intake / *Söömus*:				
Ration dry matter, kg/day *Ratsiooni kuivaine, kg/päevas*	17.2±3.1	17.0±3.3	17.0±3.06	17.1±1.5
Silage dry matter, kg/day *Silo kuivaine, kg/päevas*	13.8±3.0^{d}	11.5±2.6	9.8±2.9	9.0±1.4^{a}
Concentrate dry matter, kg/day *Jõusööda kuivaine, kg/päevas*	3.4±0.1^{bcd}	5.5±0.7^{acd}	7.2±0.9^{ab}	8.1±1.0^{ab}
ME, MJ	168.3±28.8	174.0±32.3	180.3±35.9	183.9±14.5
DMI per 100 kg live mass, kg *Kuivaine söömus 100 kg elusmassi kohta, kg*	3.49±0.5	3.48±0.7	3.48±0.8	3.49±0.4
NDF per 100 kg live mass, kg *NDF 100 kg elusmassi kohta, kg*	1.18±0.2	1.14±0.2	1.12±0.3	1.11±0.2
ME, MJ/kg dry matter *ME, MJ/kg kuivaine kohta*	9.8±0.1^{bcd}	10.3±0.1^{acd}	10.6±0.2^{ab}	10.8±0.2^{ab}
Milk production, kg/day *Piimatoodang, kg/päevas*	23.0±2.7	23.3±3.9	22.7±3.3	21.0±2.7
Milk composition: *Piima koostis*:				
Fat, % / *Rasv, %*	4.55±0.9	4.55±0.7	4.46±1.3	3.61±0.8
Protein, % / *Valk, %*	3.23±0.3	3.31±0.5	3.32±0.6	3.20±0.3
Urea, mg/l *Karbamiid, mg/l*	373±65.6	355±72.7	333±35.4	334±57.6

The effect of barley meal on ruminal fluid characteristics

Ruminal fluid characteristics imply to intensive ruminal fermentation of carbohydrates (Table 3) in all trial variants. The increase of barley meal proportion in the ration resulted in the increase of total acidity of ruminal fluid, the decrease of pH value and the proportion of acetic acid in the total quantity of acids. The ratio of acetic and propionic acid decreased as well, explaining why milk fat content dropped in the case barley meal proportion in the ration was high.

Table 3. The effect of barley meal on ruminal fluid characteristics
Tabel 3. *Odrajahu mõju vatsavedeliku näitajatele*

Item Näitaja	Barley meal proportion of energy need, % *Odrajahu osatähtsus energiatarbest, %*			
	25 a	40 b	55 c	70 d
Total acidity, mmol/dl *Üldhappesus, mmool/dl*	14.45±0.9^{cd}	17.39±2.6^{cd}	23.16±2.6^{ab}	28.28±3.2^{ab}
pH	6.02±0.1^{cd}	5.85±0.1^{cd}	5.54±0.1^{abd}	5.28±0.0^{abc}
Acetic acid (C_2), % *Äädikhape C_2), %*	57.81±3.4^{c}	57.63±1.0^{c}	51.60±0.8^{ab}	48.85±5.6
Propionic acid (C_3), % *Propioonhape (C_3), %*	20.74±1.2	21.42±1.1	22.17±3.7	24.75±4.4
Butyric acid (C_4), % *Võihape (C_4), %*	13.42±3.3	14.48±3.2	18.49±2.6	16.66±3.7
$C_2 : C_3$	2.79±0.3^{d}	2.69±0.2^{d}	2.33±0.4	1.97±0.3^{ab}
NH_3 – N mg/dl	38.33±14.0^{d}	29.11±10.2^{d}	16.04±1.9	9.40±4.5^{ab}

When the proportion of barley meal in the ration increased, the content of ammonium nitrogen in ruminal fluid and the ration protein content decreased, but that did not become the limiting factor of the microbial protein synthesis in the rumen. According to the investigations by Roffler and Satter (1975), the quantity of ammonium nitrogen in ruminal fluid should be 4…5 mmol/l for the maximum microbial protein synthesis.

The effect of barley meal on PD excretion and microbial-N synthesis

In the case barley meal proportion in the ration was increased the content of NFC and DOMR increased as well, whereas the protein content decreased. Due to this the daily urine excretion volume decreased and the urine nitrogen content increased. The increase of barley meal proportion in the ration resulted in a substantial increase of the urine allantoin content, calculated PD excretion with the urine and the absorption of microbial PD (Table 4).

The question, why the volume of daily excreted urine dropped when barley meal proportion in the ration increased, can be well explained by the results of the trial made by Moscardini *et al*. (1998) who found out that the volume of daily excreted urine of the cows was correlated with the amount of rumen degradable protein in the ration.

The daily urinary allantoin excreation correlates with DM intake (r=0.553; P<0.05), ME intake (r=0.665; P<0.01), NCF intake (r=0.662; P<0.01), DOMR content in the ration (r=0.664; P<0.01), urinary N content (r=0.562; P<0.05) and daily N excretion (r=0.601; P<0.01).

Table 4. The effect of barley meal on purine derivative excretion
Tabel 4. *Odrajahu mõju puriinterivaatide eritumisele*

Item *Näitaja*	Barley meal proportion of energy need, % *Odrajahu osatähtsus energiatarbest*, %			
	25 a	40 b	55 c	70 d
Crude protein intake, g/day *Toorproteiini söömus, g/päevas*	3543±752	3318±718	3174±760	3110±209
NFC intake, kg/day *NFC söömus, kg/päevas*	7.7±1.0	8.5±1.5	9.0±1.6	9.4±1.0
DOMR, kg/day *DOMR, kg/päevas*	7.0±1.2	7.2±1.3	7.3±1.4	7.5±0.6
Urine volume, l/day *Uriini kogus, l/päevas*	38.1±11.2^{d}	27.4±8.0	27.1±7.9	24.3±1.6^{a}
Total urine-N, g/l *Uriini üld-N, g/l*	7.6±0.8^{d}	8.3±0.9	8.1±0.8	9.5±1.2^{a}
Urine urea, mmol/l *Uriini karbamiid, mmool/l*	197.9±44.9	176.8±29.0	181.3±31.1	207.6±49.4
Urine allantoin, mg/l *Uriini allantoiin, mg/l*	1022±277^{cd}	1292±146^{d}	1598±213^{a}	1901±168^{ab}
Urinary N excretion, g/day *Uriin-N eritumine, g/päevas*	296±95	235±82	229±80	236±29
Urinary urea-N excretion, g/day K*arbamiid-N eritumine uriiniga, g/päevas*	213.1±84.5	137.0±47.1	139.0±48.1	139.5±25.0
Urinary allantoin excretion, mmol/day *Allantoiini eritumine uriiniga, mmool/päevas*	232.1±28.7	225.6±81.6	270.6±70.4	290.9±9.9
Urinary PD excretion, mmol/day *PD eritumine uriiniga, mmool/päevas*	273.1±33.8^{d}	265.4±96.0	318.4±82.8	342.4±23.3^{a}
Absorbed microbial PD, mmol/day *Imendunud mikroobseid PD, mmool/päevas*	274.2±37.1^{d}	265.9±113.1	327.7±97.6	355.5±28.0^{a}

On the basis of the trial results it can be concluded that in all trial variants the synthesis of ruminal microbial-N was lower than predicted, considering ME intake or the amount of DOMR in the ration (Table 5). Computed by purine derivatives 0.98…1.24 g ruminal microbial-N was synthesized for MJ^{-1} ME and

23.24…29.83 g for kg^{-1} DOMR which is significantly less than calculated by ARC (1984) norms. The results coincide well with the data by Thomas and Rae (1988) who also proved that alfalfa silage protein quality is low concerning the protein metabolism of the ruminants.

Table 5. Microbial-N synthesis according to barley meal proportion in ration and computed methods, g/day
Tabel 5. *Mikroobse N süntees olenevalt odrajahu osatähtsusest ratsioonis ja arvutuse meetodist, g/päevas*

Computed method *Arvutuse meetod*	Barley meal proportion of energy need, % *Odrajahu osatähtsus energiatarbest, %*			
	25 a	40 b	55 c	70 d
PD basis *PD kaudu leitud*	172.6±23.4[d]	167.3±71.1	206.2±61.4	223.7±17.7[a]
DOMR basis *DOMR kaudu leitud*	222.2±37.0	229.3±42.2	234.5±46.0	240.9±20.5
ME intake basis *ME söömuse kaudu leitud*	235.6±40.4	243.6±45.3	252.4±50.3	257.5±20.3

Conclusions

The increase of barley meal proportion in the cows ration results in the increase of microbial protein synthesized in the rumen whereas the additional feeding of barley meal does not synchronize sufficiently energy and protein hydrolysis in the rumen and it does not maximize the microbial protein synthesis in the case alfalfa-rich ration is used.

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