Effect of Dietary Protein on Rumen Fiber Kinetics and Fiber Digestion in Dairy Cows

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ABSTRACT: In a pilot study the effect of supply of rumen degraded protein on rumen fiber kinetics was investigated in four rumen cannulated Holstein cows. The cows were fed ad libitum with the same total mixed ration low in degradable protein. Feed intake varied from 16 to 20 kg DM/d. Two cows were supplemented with urea (150 and 50 g/d, respectively), to increase rumen protein balance (PBV). Faecal excretion was calculated using feed indigestible NDF (INDF) as marker and rumen pools were determined from total evacuations. Obtained PBV-level was -13 g/SFU (Scandinavian Feed Unit) for the unsupplemented cows and –4 and +13 g/SFU for the supplemented cows; and mean retention time (MRT) of INDF and fractional rate of degradation (k_d) of digestible NDF were: MRT = 40 h, 37 h and k_d = 0.023 h^{-1}, 0.040 h^{-1} for the two supplemented cows; and MRT = 26 h, 25 h and k_d = 0.043 h^{-1}, 0.034 h^{-1} for the two unsupplemented cows. Based on these four observations, urea supplementation at the actual PBV levels seemed not to have a positive effect on fractional rate of fiber degradation, probably due to the high PBV values for the unsupplemented cows..

Key words: Dairy cows, PBV-level, Fiber digestion

Introduction

According to Danish requirements for dairy cows a feed ration must contain minimum 260 g cell wall carbohydrates per Scandinavian feed unit (SFU) to prevent rumen dysfunction. Fiber is a common term for cell wall carbohydrates, which consist of cellulose, hemicellulose and lignin. The fiber fraction cannot be digested by the enzymes produced by the cow itself, and must be broken down by rumen fermentation. To analyze for cell wall carbohydrates the van Soest analysis is commonly used.

Forages are the most important source of dietary fiber, and therefore important an energy source for dairy cows. Forages are assigned a fixed value of net energy based on chemical composition and digestibility. However, the utilization of cell wall carbohydrates in the rumen is subject to multiple interactions from feeding level, concentrate: forages ratio and rumen environment ect. The actual digestibility of fibers is dependent on e.g. microbial environment and feed retention time in the rumen. Rumen environment affects the fractional rate of degradation and the daily DM intake influence the fractional rate of passage; and the digestibility is the result of the competition between the fractional rate of degradation and the fractional rate of passage out of the rumen.

In animal production it is beneficial for both the producers and the environment if the administration of nutrients to the animals is according to the animals demand. However, in high yielding dairy cows only about 25% of the nitrogen (N) intake is used for meat or milk production (Børsting et al., 2003). Rumen degradable protein is essential for rumen metabolism and therefore, the Nordic protein evaluation system operates with a measurement for feeds that estimates the protein balance in the rumen (PBV). The PBV is calculated as the difference between degraded feed protein and microbial protein synthesis without including the recirculation of nitrogen to the rumen. Børsting et al. (2003) argued that maximum milk yield was reached at a protein balance in the rumen of zero. This implies that any excess of rumen degradable protein will be excreted through the urine as a potential source of pollution to the environment. Insufficient supply of dietary N inhibits the efficiency of rumen microbes and eventually decreases fiber utilization. Due to the importance of rumen available protein, it is interesting to establish the minimum PBV-level without causing a decrease in fiber digestion. The present pilot study hypothesized that insufficient available dietary N would cause a decreased feed intake and NDF digestibility. The specific objectives were: 1) to analyze the chemical composition of the feed ration, 2) to analyze the digestibility of NDF by in situ and in vivo techniques and 3) to determine the effects of low PBV-level in the ration on fiber digestibility.

Materials and Methods

Experimental design

This pilot study was designed to investigate whether the PBV level had an effect on feed intake and NDF digestibility in dairy cows. The experimental design is illustrated in Table 1.

Animals

The present experiments complied with the guidelines of the Danish Ministry of Justice (Act no. 726, 1993) with respect to animal experimentation and care of animals under study.

Four rumen cannulated Danish Holstein cows (no. 1, 2, 3, 4), of which three of the cows were duodenum and ileum fistulated (1, 2, 4) were used in the present experiment. Two of the cows were in early and first lactation and the remaining two cows were either dry or in late lactation. The four cows had an average weight of 683 ± 103 kg and were all housed under the same conditions in tie stalls and on rubber mats.

Table 1. The experimental design of the present experiment including the two treatments.

	Cows			
	1	2	3	4
Urea, g/d	0	150	0	50
Lactation stage	Dry	Early	Early	Late
PBV, g/SFU	-26	-2	-26	-2

PBV: expected protein balance in rumen; SFU: Scandinavian feed units

Feeds

A total mixed feed ration (TMR) was fed ad libitum two times daily at 7.30 in the morning and 16.30 in the afternoon. The feed composition is listed in Table 2.

Table 2. The composition of the TMR as percent of DM.

Feed	% of DM
Rapeseed cake	20.6
Ground barley	9.4
Dried beet pulp	5.1
Maize silage	63.6
Minerals (Type 1)	1.3
Total	100

In addition, the TMR was daily supplemented with commercial vitamin mix "rød Solitren" 30 g/d (Løven Agro, Denmark). The TMR had the characteristics listed in Table 3, which was based on table values for concentrates and chemical analysis of the maize silage. The cows had free access to water.

Table 3. Expected characteristics of the TMR ration.

Unit	Feed ration
Energy, kg DM/SFU	1.01
PBV, g/SFU	-26
Chewing time, min/SFU	22

SFU: Scandinavian feed unit; PBV: Protein balance in rumen

In addition to the feed ration, two of the cows were daily in connection to feedings supplemented with a fixed amount of urea (2x75 g to cow no. 2 and 2x25 g to cow no. 4) to increase the expected PBV level to –2 g PBV/SFU. The urea supplementation was based on an expected feed intake of 18 SFU for the early lactating cows and 6 SFU for the late/dry lactating cows.

Sample collection

Over a course of six days the daily water intake and feed intake was recorded. Feed intake was calculated based on daily recordings of feed offered and residues. Five faecal samples of approximately 100 g were collected on day two to day four in the sampling periods, and subsequently pooled. Total rumen evacuations were performed once on each cow at 12.00 on the second day of sample collection. One sample of total 500 g was compiled from the solid and fluid fractions of the rumen content after mixing the individual fractions. On the sixth day of sampling approximately 500 ml rumen liquid was collected every hour for 9 h starting before feeding in the morning and ended before feeding in the afternoon on the last day of sampling. The pH of the rumen fluid was measured immediately after collection and the remaining were stored at -20°C and analyzed for NH_3 using Kjeldahl.

Effective rumen protein and NDF degradation, in situ technique

On the third day of sampling the effective rumen protein and NDF degradation was measured using series of nylon bags incubation. The nylon bag residue was analyzed for NDF content using the ANKOM methods. The true total digestibility of protein in the ruminal gastrointestinal tract was estimated using the mobile nylon bags. The procedures are described by Hvelplund et al. (2000a).

Digestibility, in vivo technique

Long term (168 h) incubations were performed in order to determine content of indigestible NDF (INDF). The feed, fecal and rumen samples were freeze-dried and milled to 1.5 mm. One g of sample was placed in each 6 nylon bags (2 x feed; 2 x faecal and 2 x rumen content) and put into therumen of a standard cow. The bags were removed after 168 h incubation and analyzed for NDF using the ANKOM methods. The following procedure was used to calculate the in vivo digestibility of NDF: A part of NDF is indigestible (INDF) and the rest can potentially be digested (DNDF) by the cow. Using the INDF as an internal marker, the in vivo digestibility of e.g. NDF can be calculated based on determining INDF:NDF ratio in feed and faeces respectively.

The ratio between INDF and NDF in the samples was calculated as: 100 % * $INDF_{residue\ 168h}$ / NDF_{0h}, as this method accounts for initial particle loss from the bags. Hereafter the amount INDF fed to the cow is calculated from the known NDF in the feed ration: NDF kg_{fed} * % INDF of the NDF fed = INDF kg_{fed}. It is assumed that the amount of INDF fed to the cows can be recovered in faeces.

The faecal flow of NDF can subsequently be calculated from intake of INDF and INDF:NDF in faeces. The in vivo digestibility of NDF in the rumen can be calculated according to the equation: (NDF_{intake} – $NDF_{excreted}$) / NDF_{intake} * 100 % = NDF digestible (%)

The same procedure with INDF as a marker is used when the digestibility of OM, protein and DNDF is calculated.

Rumen kinetics, in vivo technique

From the NDF analysis of rumen content the ruminal fractional passage rate of NDF can be estimated; however, a few assumptions have to be made. The rumen pool and fractional passage rate (k_p) are assumed to be constant (first order kinetics). Animals are assumed to be in steady state e.g constant feed intake and the fractional rate by which the feed enters the rumen and exit the rumen by either digestion or passage is the same; hereby, the rumen pool becomes constant. It is assumed that 95% of the total amount of NDF digested is degraded in the rumen and 5% is degraded in the hindgut. The rumen fractional passage rate (k_p) is calculated according to the equation: ((estimated rumen out flow, kg/d / rumen pool, kg) / 24h)*100 % = %/h. The rumen pool of NDF is known from analysis of total rumen content. The fractional rate for

NDF entering the rumen (k_i) can be calculated from the feed analysis and the rumen pool; however, the fractional rate of degradation (k_d) is unknown. The fractional rate of degradation is calculated based on the amount of NDF degraded in the rumen (intake - estimated rumen outflow) and rumen NDF pool. The same procedure and assumptions are used to calculate the passage rate for DNDF. Mean retention time (MTR) is calculated as MRT (h) =1/k_p.

Results

The feed ration used contained on a DM basis 94.5% organic matter (OM), 13.7 % crude protein (CP), 17.2% crude fiber, 35.3% NDF, 9.3% INDF, 26.0% DNDF and 6.4% crude fat. The AAT and PBV values were calculated to be 86 g/SFU and -13 g/SFU and there were 0.95 SFU per kg DM. In Table 4 is listed the daily feed intake for each cow together with the PBV-level.

Table 4. Daily feed intake (kg) and observed PBV level.

	Cow			
	1	2	3	4
PBV, g/SFU	-13	13	-13	-4
SFU	14	16	18	15
DM	16	17	20	17
Urea, g	0	150	0	50
OM	15.1	16.5	18.9	16.1
CP	2.2	2.4	2.7	2.3
NDF	5.6	6.1	7.0	6.0
INDF	1.48	1.61	1.84	1.58
DNDF	4.14	4.52	5.17	4.42

The observed values for protein degradation at different times are illustrated in Figure 1. The observed values do not indicate any treatment effect.

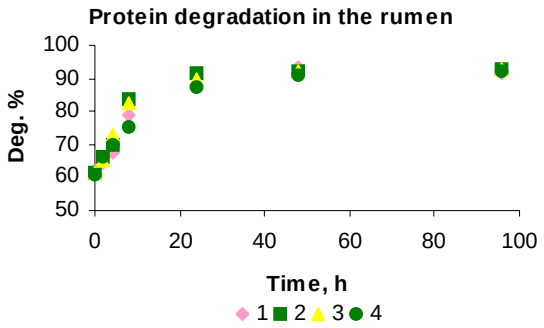

Figure 1. Rumen protein degradation profile for the four cows. The plots are observed values.

In Table 5 and Table 6 are listed the rumen pools and faecal flows after chemical analysis of rumen contend and faeces. Despite only minor differences were found in feed intake, rumen pool of NDF varied from 4.2 to 7.7 kg and total content varied from 56.9 to 80.8 kg. Faecal flow seemed to be highest for cow no. 3 in accordance with the highest feed intake.

Table 5. Rumen pools (kg).

	Cow			
	1	2	3	4
PBV, g/PBV	-13	13	-13	-4
Total	56.9	80.8	65.1	64.6
DM	7.9	12.9	9.6	9.3
OM	7.3	12.0	8.9	8.7
N	0.20	0.31	0.24	0.22
NDF	4.2	7.7	5.5	5.5
INDF	1.6	3.0	1.9	2.5
DNDF	2.6	4.7	3.6	3.0

Table 6. Faecal flows (kg).

	Cow			
	1	2	3	4
PBV, g/PBV	-13	13	-13	-4
DM	6.1	6.1	8.2	5.4
OM	5.3	5.5	7.4	4.8
N	0.17	0.16	0.22	0.14
NDF	2.9	3.3	3.9	2.9
INDF	1.5	1.6	1.8	1.6
DNDF	1.4	1.6	2.1	1.3

The results of the apparent in vivo total digestibility of NDF, OM and protein as estimated by INDF are listed in Table 7. Table 7 also includes the in vivo digestibility of DNDF.

Table 7. The apparent in vivo total digestibility of different nutrient components in the feed ration.

	Cow			
	1	2	3	4
PBV, g/PBV	-13	13	-13	-4
OM	65	67	61	70
NDF	48	46	44	52
DNDF	66	64	60	70
Protein	52	58	49	62

The observed values for NDF degradation at different times are illustrated in Figure 2. The observed values have not been corrected for loss of particles. The results do not indicate any treatment effect.

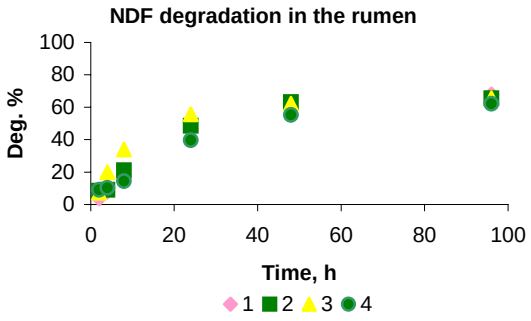

Figure 2. The rumen degradation of NDF at different times for the four cows. The plots are observed values.

Digestibility of feed in the rumen is a result of a balance between fractional rate of degradation (k_d) and fractional rate of passage (k_p) of particles out of the rumen (Hvelplund et al., 2000b), which are listed in Table 8 and illustrated in Figure 3.

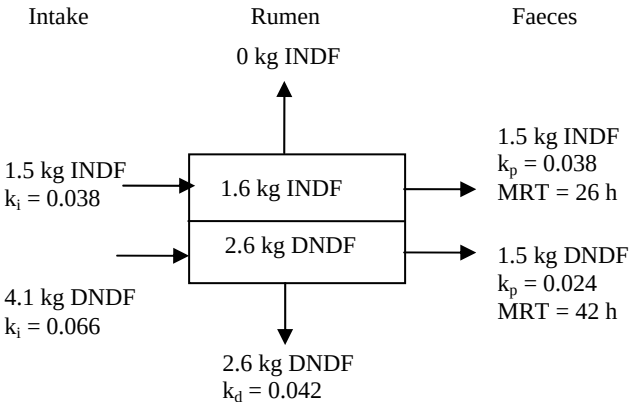

Figure 3. Illustration of rumen fiber kinetics for cow no 1.

Table 8. The in vivo fractional passage rate of NDF and INDF (h^{-1}) and mean retention time (h).

	Cow			
	1	2	3	4
PBV, g/PBV	-13	13	-13	-4
k_p NDF	0.030	0.018	0.031	0.023
k_p INDF	0.039	0.025	0.040	0.027
k_p DNDF	0.024	0.016	0.027	0.020
k_d DNDF	0.043	0.023	0.034	0.040
MRT_{NDF}	33	56	32	43
MRT_{INDF}	26	40	25	37
MRT_{DNDF}	42	63	37	53

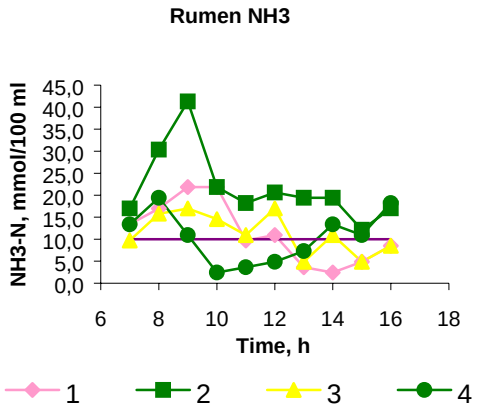

Figure 4. The rumen NH_3 concentration measured over 9 h.

Figure 4 illustrates the variations of rumen NH_3 concentration over 9 h during the day. The NH_3 concentration increase weakly right after feeding and then levels out until next feeding. Only cow no. 2 of the two urea-supplemented cows peaks right after feeding.

Discussion

Forages fiber makes up a substantial part of the net energy for dairy cows and in contradiction to other feed fractions; fiber digestibility is highly influenced by feeding level and rumen function. The maximum digestibility of a feed is related to the chemical composition of the plant whereas the actual digestibility is dependent on ruminal function and feed intake (Hvelplund et al., 2000b).

The expected PBV-level of -26 g/SFU was not reached in the present experiment, which probably can explain the absence of a negative effect on feed intake. It is well known, that insufficient supply of N to the rumen microbes decreases fiber utilization (Heldt et al., 1999). The fractional passage rate out of the rumen has previously been found to be independent of the N supply to the rumen microbes (Weisbjerg, 1999) but positively related to DM intake (Stensig et al., 1997), which is comparable to the present experiment. The daily DM intake for cow no. 3 was 15% higher than cow no. 2 per day, which resulted in an increased k_p for NDF from 0.018 h^{-1} to 0.031 h^{-1} and the correspondent MRT decreases from 56 h to 32 h. Increased rumen fractional passage rate is negative correlated with digestibility and digestibility of NDF decreased from 44% to 43% when comparing the same two cows.

The fractional rate of degradation is dependent on retention time in the rumen (Weisbjerg, 1999), which allows the microorganisms to digest the cell wall carbohydrates. This is comparable to the present experiment except for cow no. 2, which has the highest MRT and the lowest k_d for DNDF. The fractional rate of degradation however, is also dependent on an efficient rumen microflora; therefore, the present experiment expected to find a higher fractional degradation rate for the urea-supplemented cows. For PBV= 0 the rumen NH_3 concentration is 10-15 mmol/100 ml. This indicates that rumen microbes in the present experiment are not lacking N, perhaps due to N recirculation (Weisbjerg, 1997).

In conclusion, the present pilot study did not achieve the expected low PBV-level, which influenced the results throughout the experiment. A negative effect due to PBV-level was not found on feed intake, rumen and total fiber digestibility or rumen fiber kinetics. The rumen NH_3 concentration was above 10 mmol/100ml, which indicate that rumen microbial synthesis is not limited by rumen degradable protein due to a sufficient recycling to the rumen across the rumen wall and by saliva.

References

Børsting, C. F., T. Kristensen, L. Misciattelli, T. Hvelplund, M. R. Weisbjerg. 2003. Reducing surplus from dairy farms. Effects of feeding and management. Livest. Prod. Sci.83: 165-178.

Heldt, J. S., R. C. Cochran, C. P. Mathis, B. C. Woods, K. C. Olson, E. C. Titgemeyer, T. G. Nagaraja, E. S. Vanzant and D. E. Johnson. 1999. Effects of level and source of carbohydrate and level of degradable intake protein on intake and digestion of low-qulity tallgrass-prairie hay by beef steers. J. Anim. Sci. 77: 2846-2854.

Hvelplund, T. and M. R. Weisbjerg. 2000a. *In situ* techniques for the estimation of protein degradability and postrumen availability. Forage evaluation in ruminant nutrition. D. I. Givens, E. Owen, R. F. E. Axford and H. M. Omed, eds. CABI Publishing, UK. 233-258.

Hvelplund, T. and M. R. Weisbjerg. 2000b. Feed evaluation methods: Estimation of rate of digestion and rate of passage. UZ/DIAS7DANIDA – ENRECA Project Review workshop: 10-13 January, Zimbabwe.

Weisbjerg, M. R. 1999. Cellevægskulhydrater og deres omsætning I vommen. Intern Rapport no. 118. Danmarks Jordbrugsforskning, Denmark, 3-17. (In Danish)

Weisbjerg, M. R. 1997. Recirkulering af kvælstof til vommen. Intern Rapport no. 88. Statens Husdyrbrugsforsøg, Denmark, 18-23. (In Danish)