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The Influence of the Dietary Balance Between Energy and Protein on Milk Urea Concentration. Experimental Trials Assessed by Two Different Protein Evaluation Systems

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Carlsson, J., and B. Pehrson: The influence of the dietary balance between energy and protein on milk urea concentration. Experimental trials assessed by two different protein evaluation systems. Acta vet. scand. 1994, 35, 193-205. – Twentythree dairy cows were fed rations with different proportions of energy and digestible crude protein (DCP). When the ration was balanced for energy and DCP according to Swedish standard the cows' milk urea concentration was 4.66-4.92 mmol/l (95% CI of mean). With increasing intakes of DCP, fed together with standard levels of energy, the mean milk urea concentration increased in proportion to the surplus of DCP. In contrast, the concentration of urea decreased when the cows were overfed with energy at the same time as they were underfed with protein.

When the rations were recalculated in accordance with the AAT/PBV system for dietary protein evaluation the 95% CI for the mean milk urea concentration of the cows receiving a balanced ration was 3.76-4.56 mmol/l. The concentration of urea was dependent primarily on the PBV. When the 2 protein evaluation systems were compared there was a strong correlation between PBV and DCP. Ammonia was the only constituent of the rumen whose concentration was strongly correlated with the milk urea concentration.

Taken together with earlier data the present results suggest that a milk urea concentration between 4.0 and 5.5 mmol/l should be regarded as normal at least when cows are fed conventional feedstuffs.

AAT/PBV system; feeding; rumen metabolism; milk profile test.

Introduction

It has been shown that the concentration of urea in milk varies with changes in the proportions of energy and protein in the diet of cows *(Oltner & Wiktorsson* 1983, *Kirchgessner & Kreuzer* 1985, *Hoffmann & Steinhöfel* 1990, *Gustafsson & Carlsson* 1993). *Oltner & Wiktorsson* (1983) reported that the milk urea concentration increased when cows in late lactation were overfed with protein, and decreased when they were underfed with protein, provided that there was no simultaneous overfeeding or underfeeding, respectively, with energy. They concluded that there is a close relationship between milk urea concentration and the ratio crude protein/energy in the diet. This relationship was not fully confirmed by *Carlsson & Pehrson,* who in an unpublished study found that milk urea concen-

tration also increased when high-yielding cows were overfed to an equal extent with both energy and protein. In contrast, *Hoffmann & Steinhöfel* (1990) reported that the milk urea concentration decreased when low-yielding cows were overfed with both energy and protein. One possible explanation for these conflicting results may be the different milk yields of the cows used in the different experiments. In all these trials the results were based on the digestible crude protein (DCP) system for the evaluation of the dietary protein. The present trial was also planned and carried out when the DCP system was the standard method of dietary protein evaluation used officially in Sweden. However, in 1991 a new Nordic protein evaluation system (the AAT/PBV system; *Madsen* 1985) was introduced. This system considers not only the degradability of the CP in the rumen, but also the protein synthesised by the rumen microflora, and the fraction of the dietary protein that passes through the rumen undegraded. *Ropstad et al.* (1989) found that not only the intake of DCP, but also the quantity of amino acids absorbed in the small intestine (the AAT) and the protein balance in the rumen (the PBV) significantly affected the milk urea concentration. Positive correlations between milk urea concentrations and DCP and PBV were also reported by *Gustafsson & Carlsson* (1993), but they did not find any significant correlation between milk urea and AAT.

The original aim of this study was to provide a basis for a more accurate interpretation of milk urea concentration as a biological indicator of the efficiency of practical diets, when the DCP system of protein evaluation was used. A secondary aim, which developed during the trial, was to compare the effects of using the DCP and the AAT/PBV systems on the interpretation of milk urea concentrations as a guide to the efficiency of practical diets.

Materials and methods

During the indoor period of 1990-91, 23 Swedish Red and White cows were divided into 3 groups of 6 and 1 group of five. The groups were balanced as far as possible with respect to age, stage of lactation and milk yield. The cows had all calved between 62 and 103 days before the experiment began. They were 4 to 8 years old and yielded 16 to 44 kg 4% fat-corrected milk (FCM) at the beginning of the first experimental period and 16 to 38 kg FCM at the beginning of the last experimental period. They had been tied indoors for at least a month before the first experimental period and had during that time been fed a diet balanced for energy (metabolizable energy, MJ) and DCP. The diet was composed of 2 kg hay, 6.3 kg dry matter grass silage, 2 kg dried molassed beet pulp and 150 g mineral feed, and crushed oats and a commercial protein feed according to individual milk yield. This standard diet (E_sP_s) was fed to all the groups also during the first and last experimental periods and was based on the official Swedish recommendations at that time, ie maintenance plus 5.0 MJ and 60 g DCP/kg FCM. During the other experimental periods the rations were varied. Two of the rations maintained the standard input of energy but 1 (E_sP_h) provided 300 g additional DCP and the other (E_sP_{hh}) provided 600 g additional DCP. Another ration (E_hP_l) provided an additional 25 MJ of energy but 300 g less DCP than the standard diet, and another (ElPs) provided 25 MJ less than the standard amount of energy but the standard amount of protein. Each ration was fed for 2 weeks and there was a 2 week period of transitional feeding between each experimental period. The cows had access to feed from 05.15 to 08.45h and from 12.45-17.00h and were milked 06.15-07.00h and 15.30-16.30h. The concentrates and silage were fed in 4 portions per day; hay in 2 por-

tions. The experimental design is summarised in Table 1.

In order to obtain these different balances between dietary energy and protein the rations were adjusted according to Table 2. As can be seen the cows were not given dried beet pulp separately when on diet E_lP_s. On diets E_sP_h and E_sP_{hh}, 0.9 and 1.8 kg, respectively, of soya meal was added to the ration. On diet E_hP_l the silage was eliminated from the ration and the allocation of hay was increased to 8 kg. During the other periods the planned dietary balance could be achieved by maintaining the standard amounts of hay, silage and dried beet pulp in the ration, but adjusting the amounts of oats and the commercial protein feed.

The AAT (g/kg feed dry matter, d.m.) and PBV (g/kg feed d.m.) of hay and silage were calculated from analysed values of crude protein and metabolizable energy. For oats and soya meal the analysed values of crude fat, crude fibre and ash content were also used. The rumen degradability figures and the coefficients of digestibility in these calculations were taken from feed tables *(Spörndly & Wiktorsson* 1991). The AAT and PBV values of each batch of commercial protein feed were retroactively calculated by the feed manufacturer as a sum of the values of its ingredients.

Table 1. The sequence of experimental periods, each lasting 2 weeks, during which groups of cows were fed diets containing different metabolizable energy (E) and digestible crude protein (P) balances. There was a 2 week gradual transitional period (T) between each experimental period.

Period	Group I n = 6	Group II n = 5 (4)1)	Group III n = 6 (5)1)	Group IV n= 6
1	E_sP_s	E_sP_s	E_sP_s	E_sP_s
T	↓	↓	↓	↓
2	E_sP_{hh}	E_hP_l	E_sP_h	E_lP_s
T	↓	↓	↓	↓
3	E_hP_l	E_sP_{hh}	E_lP_s	E_sP_h
T	↓	↓	↓	↓
4	E_sP_s	E_sP_s	E_sP_s	E_sP_s

1) One cow in each group excluded (see p. 196).
s = standard, h = high in energy and protein, HH = double high in protein, l = low in energy or protein. (Details of feeding in the different groups and periods are presented in Table 2).

However, the AAT and PBV values of the dried beet pulp were derived directly from feed tables *(Spörndly & Wiktorsson* 1991). The scale used to evaluate the different rations in terms of the AAT/PBV system is given in Table 3 and the nutritional composition of the constituent feedstuffs is given in Table 4.

During each experimental period rumen and

Table 2. The diets and range of daily milk yield in different groups and during different periods. (Oats and commercial protein feed for the lowest and highest yield within groups).

Diet	Group	Period	Hay, kg	Silage, kg d.m.	Dried beet pulp, kg	Soya meal, kg	Oats, kg	Commercial protein feed, kg	Yield, kg FCM
E_sP_s	I + II	1	2.0	6.3	2.0	×	2.3-8.1	0.9-6.1	18-42
E_sP_s	III + IV	1	2.0	6.3	2.0	×	1.2-8.6	0.7-6.6	16-44
E_sP_h	III + IV	2+3	2.0	6.3	2.0	0.9	0.3-6.1	2.7-6.5	18-40
E_sP_{hh}	I + II	2+3	2.0	6.3	2.0	1.8	0.0-3.7	2.1-5.6	20-36
E_hP_l	I + II	2+3	8.0	×	2.0	×	10.1-11.1	0.0-0.8	22-26
E_lP_s	III + IV	2+3	2.0	6.3	×	×	0.4-4.1	3.3-7.6	18-36
E_sP_s	I + II	4	2.0	6.3	2.0	×	2.4-5.2	1.6-5.8	20-32
E_sP_s	III + IV	4	2.0	6.3	2.0	×	1.0-6.0	2.3-7.1	16-38

Table 3. Grading of the protein level in a cow ration according to the AAT/PBV system. The official Swedish standards are 3.25g AAT × body weight $^{0.75}$ for maintenance, 40 g AAT per kg FCM and 0 to +300 g PBV in total ration *(Sporndly & Wiktorsson* 1991). Energy levels (E_s; E_h;E_l) as in Table 1. A = AAT, B = PBV, s = standard, h = high, hh = double high, l = low, ll = double low.

AAT, g per kg FCM		PBV, g in total ration	
A_{ll}:	< 36	B_l =	< 0
A_l:	36 to 38	B_s =	0 to + 299
A_s:	39 to 41	B_h =	+300 to + 600
A_h:	> 41	B_{hh}=	> +600

milk samples were taken between 9.30 and 10.30 h twice a week. The rumen samples taken by stomach tube were analysed for pH, volatile fatty acids (VFA) by gas chromatography *(Carlström et al.* 1965), amylase activity by means of a reagent kit (Phadebas®, Pharmacia, Uppsala, Sweden), ammonia by flow injection analysis *(Karlberg & Pacey* 1989), and for bacterial and infusorial counts *(Engvall* 1980). The milk samples were analysed for urea by flow injection analysis *(Andersson et al.* 1986). In addition the daily milk yield, the milk fat content, and the milk protein content were determined once a week. At the end of each week the diet of each cow was adjusted to its milk production as kg FCM, and according to the DCP system.

Feed refusals were weighed every day. One cow in group II and 1 in group III failed to eat their full ration so often that they were excluded from the experiment. A few other cows occasionally left a part of their ration. When it was considered that these refusals might affect the results, all the related analyses were excluded. One cow in group I and 1 cow in group II were excluded all the time when they were being fed diet E_hP_l. They produced more than 35 kg FCM daily and refused to eat the extreme quantity of oats necessary to obtain the dietary imbalance at that production level. At the same diet a few other analyses were also excluded because of occasional feed refusals. The number of analyses was therefore considerably reduced in the E_hP_l diet (Fig. 1). One cow in group I was excluded from the last period of feeding the standard diet because she was mistakenly fed to much protein.

Table 4. The nutritional composition of the feedstuffs (per kg dry matter). In addition, each cow received 150 g daily of a standardized mineral feed, containing vitamins. The figures for hay, silages, oats and soya meal are based on mean values from double analyses and those of commercial protein feed and dried beet pulp from the values guaranteed by the manufactures.

	MJ	DCP, g	AAT, g	PBV, g
Hay	10.5	83	74	–4
Silage A[1)]	11.4	130	72	+60
Silage B[2)]	9.7	85	68	+10
Silage C[3)]	11.1	123	72	+40
Oats	11.9	85	68	–3
Commercial protein feed[4)]	13.8	222	123	+78
Dried, molassed beet pulp	12.1	67	92	–65
Soya meal	15.0	466	163	+267

1) available during the first third of the trial
2) available during the second half of the trial
3) available between the first third and the second half of the trial
4) oil cakes/meal 44%, molasses/dried beet pulp 35%, brewer's grain 7%, wheat/wheat bran 7%, gluten 3%, minerals etc. 4%

Statistical analysis

The Statistical Analysis Systems (SAS 1993) computer program was used to analyse the results. Simple coefficients of correlation (Pearson's) were calculated and several simple and multifactorial regression models were tested with milk urea as dependent factor. Some best fitting of these models with nutri-

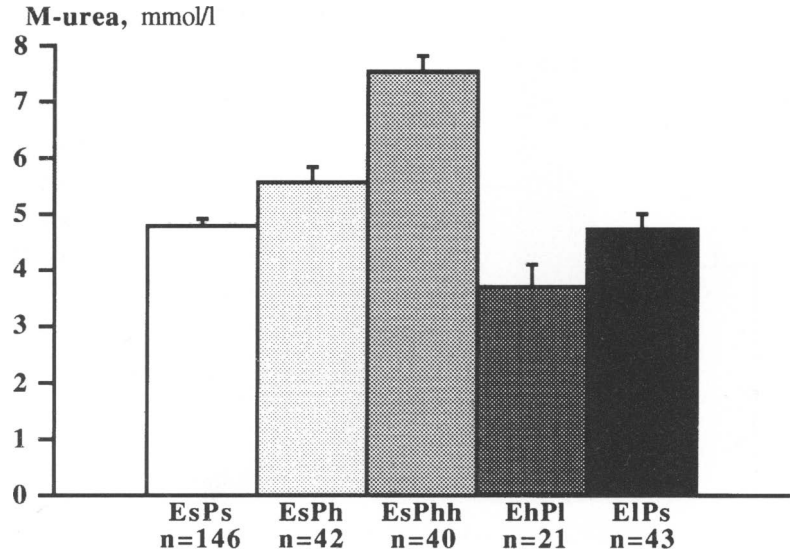

Figure 1. Mean (95% CI) concentrations of urea in milk from cows fed different rations, assessed by the DCP system for protein evaluation. E = energy; P = protein; s = standard feeding; h = moderate overfeeding; hh = heavy overfeeding; l = underfeeding; n = number of samples.

tional parameters (g DCP/kg FCM, g DCP/MJ, g AAT/kg FCM, g AAT/MJ, g PBV, MJ/kg FCM, MJ/kg feed d.m.) as independent variables are presented Table 5. Confidence intervals (CI) were based on least square means estimates. These were also used for pairwise comparisons.

Results

When the DCP system for protein evaluation was used, the 95% CI for the mean milk urea concentration of the cows fed the standard diet (E_sP_s) was 4.66-4.92 mmol/l when controlling for variation between individuals and for stage of lactation (Fig. 1). When the protein content of the diet was increased at standard feeding of energy, the estimated least square mean milk urea concentration increased approximately in proportion to the increase in dietary protein: to 5.57 mmol/l on diet E_sP_h and to 7.54 mmol/l on diet E_sP_{hh}. The mean milk urea concentration decreased

Table 5. Some of the best fitting models for multifactorial regression analysis when using milk urea concentration as dependent variable and the DCP (Model 1-2) and AAT/PBV (Model 3-4) systems for protein evaluation controlling for variation between individuals and stage of lactation.

Nutritional factors	P	r^2
Model 1:		0.74
DCP/MJ	<0.001	
MJ/kg d.m.	<0.001	
Model 2:		0.74
DCP/MJ	<0.001	
DCP/kg FCM	n.s.	
MJ/kg FCM	<0.001	
Model 3:		0.72
PBV	<0.001	
AAT/kg FCM	<0.001	
Model 4:		0.72
AAT/MJ	<0.001	
MJ/kg d.m.	<0.001	

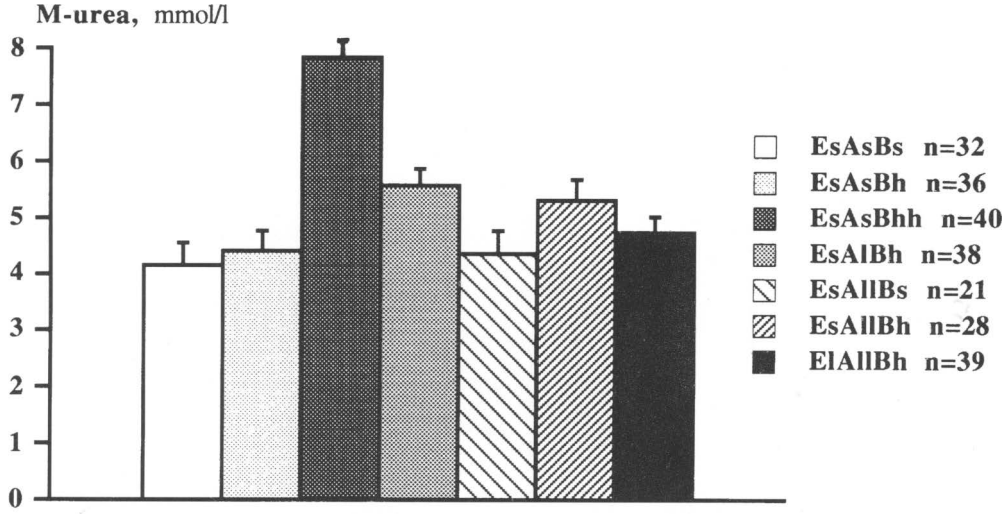

Figure 2. Mean (95% CI) concentration of urea in milk from cows fed different rations, assessed by the AAT/PBV system for protein evaluation. E = energy; A = AAT; B = PBV; s = standard feeding; h = moderate overfeeding; hh = heavy overfeeding; l = moderate underfeeding; ll = heavy underfeeding; n = number of samples.

to 3.70 mmol/l when the cows were fed excess energy and a deficiency of protein (diet E_hP_l), and didn't change when they were fed too little energy but a standard amount of protein (4.72 mmol/l; diet E_lP_s). The mean urea values of all diets, except E_lP_s, were significantly ($P<0.001$) different from the E_sP_s diet at two-part comparisons.

When the rations were recalculated according to the AAT/PBV system, using the scales for the evaluation of AAT and PBV given in Table 3 as a basis, only 1 ration had the recommended balance between energy, AAT and PBV ($E_sA_sB_s$). Six unbalanced rations could be defined (Table3) with more than 20 observations for each. The mean (95% CI) concentration of urea in the milk of the cows fed the fully balanced ration was 3.76-4.56 mmol/l (Fig. 2). The mean urea concentrations in the milk of the cows fed all the other diets were significantly ($P<0.05$) different from this value except for $E_sA_sB_h$ and $E_sA_{ll}B_s$.

When using milk urea concentration as dependent variable the 2 best fitting models containing one nutritional factor and controlling for variation between individuals and for stage of lactation in the DCP system were for g DCP/MJ ($r^2 = 0.67$) and for g DCP/kg FCM ($r^2 = 0.72$). In the AAT/PBV system the 2 best fitting corresponding models were for g PBV ($r^2 = 0.70$) and for MJ/kg feed d.m. ($r^2 = 0.66$). The r^2-values in similar models were 0.58 and 0.39 for g AAT/MJ and g AAT/kg FCM, respectively. Milk urea concentration as dependent on only individual cow and stage of lactation gave an r^2-value of 0.27. At simple correlation tests between protein evaluation systems r-values of 0.91 and 0.79 were found between g DCP/MJ and g PBV and between g DCP/kg FCM and g PBV, respectively.

Table 5 illustrates the best fitting models for multifactorial regression analyses controlling for variation between individuals and stage of lactation. In the DCP system the r^2-value

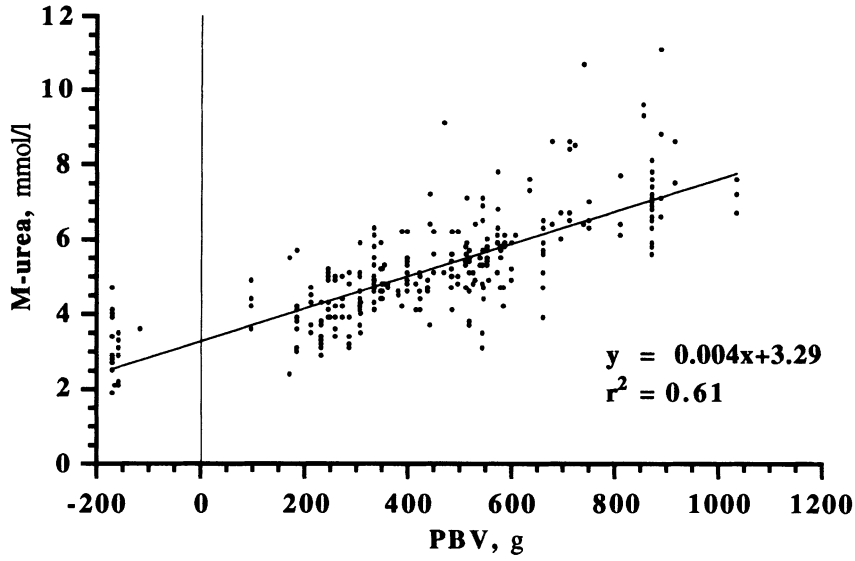

Figure 3. The relationship between milk urea concentration and the protein balance in the rumen (PBV). Number of samples = 292.

could be increased to 0.74 when a model containing 2 nutritional factors was used by adding MJ/kg feed d.m. to g DCP/MJ. No further increase occurred with the best fitting three-factor model (r^2 = 0.74), which included g DCP/MJ, g DCP/kg FCM and MJ/kg FCM. In the AAT/PBV system the r^2-value was nearly identical (0.72) when the factor g AAT/kg FCM was added to g PBV. No further increase was reached after adding more factors. It was also possible to construct multifactorial models that explained milk urea equally well without including PBV. The best fitting model was for g AAT/MJ and MJ/kg feed d.m. as independent variables (r^2 = 0.72). However, when evaluating all these multifactorial models it must be realized that most of these independent factors were related to the dietary protein.

The strong relationship between milk urea and g PBV is also illustrated in Fig. 3.

Of the measurements made on ruminal fluid, only the concentration of ammonia had a strong correlation with milk urea concentration (r = 0.48; Fig. 5). However, a number of significant correlations were found between the different measurements. There were strong positive correlations between the VFAs, (acetic, propionic and butyric acids), with r values between 0.57 and 0.72, strong negative correlations between pH and the VFAs (r = –0.59 to –0.76), moderate positive correlations between ammonia and the VFAs (r = 0.25 to 0.34), moderate positive correlations between the total number of bacteria and the VFAs (r = 0.21 to 0.30), and weak negative correlations between pH and the total number of bacteria (r = –0.19), the number of protozoa (r = –0.24) and the amylase activity (r = –0.20).

When the DCP system for protein evaluation was used, the 95% CI for the mean values of the different ruminal parameters in the balanced group of cows (EsPs) were (Fig. 4): for

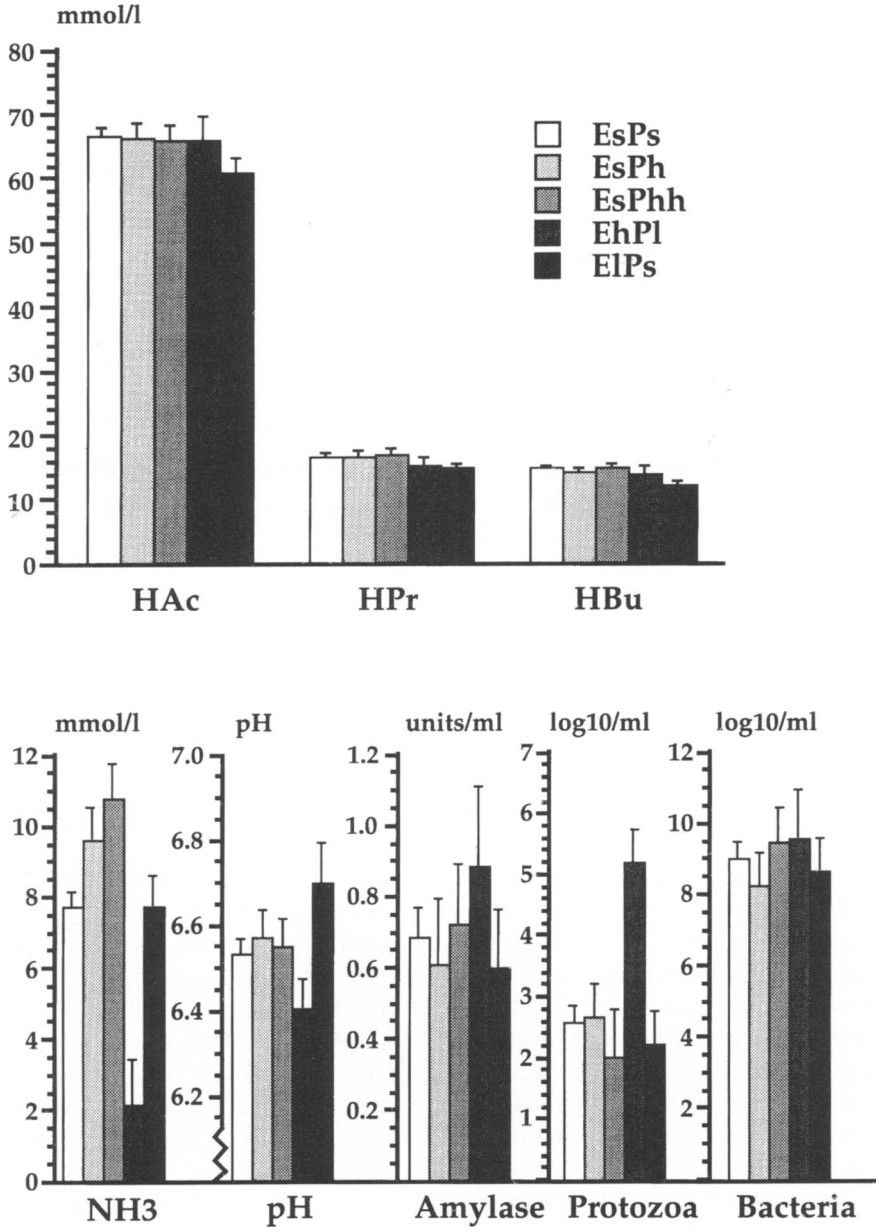

Figure 4. a (upper panel) and b (lower panel). Mean (95% CI) of different rumen fluid parameters in cows fed different rations assessed by the DCP system for protein evaluation. E = energy; P = protein; s = standard feeding; h = moderate overfeeding; hh = heavy overfeeding; l = moderate underfeeding; HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; NH_3 = ammonia. n = number of samples.

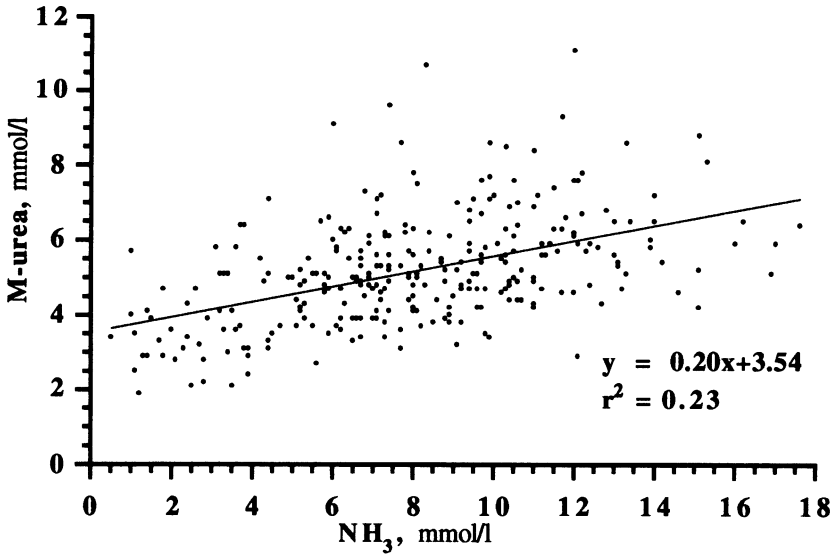

Figure 5. The relationship between milk urea concentration and the ammonia (NH3) concentration in the rumen. Number of samples = 292.

pH 6.50-6.56, for NH_3 7.28-8.10 mmol/l, for HAc 65.5-67.7 mmol/l, for HPr 16.3-16.9 mmol/l, for HBu 14.4-15.0 mmol/l, for total number of bacteria 8.6-9.4 log^{10}/ml, for infusoria 2.4-2.8 log^{10}/ml and for amylase activity 0.60-0.76 units/ml. The most notable deviations were the lower VFA concentrations in the group underfed with energy at protein balance (E_lP_s), the different pH's in the 2 groups with a divergent energy supply (E_hP_l and E_lP_s), the much lower NH_3 concentration in the protein deficient group (E_hP_l), the higher NH_3 concentration in the groups overfed with protein and the differences in the number of protozoa between the group with excess energy (E_hP_l) and the others.

As mentioned above the concentrations of ammonia in rumen fluid was correlated to urea in milk (see also Fig. 5). None of the other ruminal parameters were found to be of interest for explaining the total variation of milk urea when tested in multiple regression models.

Discussion

It is generally accepted that the urea in milk is derived from ruminal ammonia, after it has been converted to urea in the liver. The relationship ($r^2 = 0.23$) between the concentration of ammonia in the rumen and the concentration of urea in milk (Fig. 5) is therefore to be expected. When controlling for variations between individuals and for stage of lactation the r^2 value increased to 0.46, a value similar to that found by Ropstad et al. (1989; r = 0.74). When the cows were fed a standard amount of energy and an excess of DCP, the milk urea concentration increased (Fig. 1). These results, together with those of an own unpublished study including also a still higher excess of DCP (standard energy and 900 g additional DCP), indicate that each additional 60 g of

DCP fed to cows receiving only their requirement of energy will increase milk urea concentration by from 0.2 to 0.3 mmol/l. This increase is presumably due to the degradation in the rumen of surplus DCP to ammonia which was unavailable for the synthesis of microbial protein. According to the results of *Hoffman & Steinhöfel* (1990), cows receiving more than their requirement of protein should be expected to make more efficient use of the protein for microbial synthesis if they are also fed more than their requirement of energy. However, our data obtained from an unpublished experiment showed that the concentration of urea in milk increased when cows fed a diet balanced according to their requirements for energy and DCP were fed extra energy and protein. If it is supposed that the ruminal flora of intensively fed cows are less able to use additional energy for the synthesis of microbial protein, then 1 possible reason for these conflicting results might be the much higher milk yields of the cows used in the authors' experiments.

The low milk urea concentrations observed in the cows fed too little DCP was also expected, and in accordance with earlier reports *(Oltner & Wiktorsson* 1985).

The important difference between the 2 protein evaluation systems is illustrated by the fact that the cows which were overfed with protein according to the DCP system (i.e. those fed diets E_sP_h and E_sP_{hh}) were found to have been fed only the standard amount of AAT (i.e. A_s diets) when the AAT/PBV system was applied; as a result a majority of the cows were in reality underfed with AAT (Fig. 2). The experiment was therefore unable to evaluate the effect of overfeeding cows with AAT. However, the fact that the addition of g AAT/kg FCM to g PBV in model 3 (Table 5) had a significant effect on milk urea – even if the increase of the r^2-value was just from 0.70 to 0.72 – may indicate that some part of what is theoretically defined as AAT in reality was excreted as urea in the milk. This urea may be derived either from ammonia produced in the rumen or from the endogenous metabolism of an excess of absorbed amino acids; however, since none of the rations contained any surplus of AAT, the first suggestion seems more likely. Also *Ropstad et al.* (1989) observed a correlation between the milk urea concentrations of individual cows and their intakes of AAT, but *Gustafsson & Carlsson* (1993) failed to find such a correlation in a study based on the measurement of urea concentrations in samples of bulk milk. However, in the latter study the cows were fed more energy than their standard requirements and might therefore have been better able to synthesise microbial protein from ammonia. Further work is necessary to establish whether a possible ruminal release of ammonia from AAT can be reduced or eliminated by increasing the allocation of energy for milk production to more than the 5.0 MJ/kg FCM fed to the cows in the present study.

Considering both systems of protein evaluation PBV was the parameter which was most significantly correlated with milk urea concentration, in agreement with the results of *Ropstad et al.* (1989) and *Gustafsson & Carlsson* (1993). This finding is not surprising for 2 reasons; first because PBV is a measure of the oversupply of rumen digestible crude protein in relation to the amount of energy available to the rumen microbes; and secondly because the term DCP includes rumen undegradable protein and might therefore be expected to have a lower correlation with milk urea concentration than PBV. Indeed, the fact that DCP includes the rumen undegradable protein makes the comparatively high r^2-values for g DCP/MJ and g DCP/kg FCM with milk urea as dependent variable rather surprising.

One possible reason is that 3 of the 5 groups were overfed with DCP in relation to their energy intake (Fig. 1). Therefore, a majority of the samples were from cows which could be expected to have been less than optimally efficient in their metabolism of nitrogen. Moreover, neither *Higginbotham et al.* (1989) nor *Roseler et al.* (1993) found any differences between the milk urea concentrations of cows fed diets containing either large or small amounts of what was considered to be easily degradable protein, indicating that the theoretically clear difference between rumen degradable and undegradable protein in practice can be quite indistinct.

Most of the results from the ruminal measurements were expected: the high ammonia concentration in the high protein groups, the low ammonia concentration in the low protein group, the low concentrations of VFAs in the energy deficient group, the lower pH in the group that was overfed with energy, the negative correlation between pH and VFAs, and the higher numbers of protozoa in the excess energy group. The higher amylase activity and the negative correlation between amylase activity and pH in the group overfed with energy were also expected because this group received larger amounts of starch-rich grain than the others. The positive correlations between ruminal ammonia and the 3 VFAs ($r = 0.25$ to 0.34) are difficult to explain, but may in some way be related to the efficiency of activity of the ruminal flora. The negative correlation between pH and the numbers of protozoa was unexpected because *Church* (1976) observed a decrease in the numbers of protozoa at lower pHs. However, the deleterious effects of low pH on the protozoa appear to be restricted to pHs below 6.0 *(Schwartz & Gilchrist* 1975), and in the present study only a few of the ruminal samples had a pH below 6.0.

The least square mean estimated milk urea concentrations of the cows fed the fully balanced diets, defined according to the DCP or the AAT/PBV systems of protein evaluation, were 4.79 and 4.16 mmol/l, respectively. If the mean ±2 sd is used to define the normal range for individual cows, then the normal ranges of milk urea concentration would be 3.5-6.3 mmol/l in the DCP system and 3.0-5.3 mmol/l in the AAT/PBV system. However, when considering the practical significance of a "fully balanced ration", narrower ranges are probably justified for the definition of an optimal milk urea concentration. Considering the present data together with other results, which suggest that the milk urea concentrations of cows fed balanced diets may be rather higher, both with the DCP system *(Oltner & Wiktorsson* 1985, *Emanuelson et al.* 1993) and with the AAT/PBV system *(Volden et al.* 1992), it is proposed that milk urea levels between 4.0 and 5.5 mmol/l should be regarded as normal for high yielding cows when conventional feedstuffs are used, except possibly during the first month of lactation when lower values have often been reported than later in lactation *(Gustafsson et al.* 1987, *Volden et al.* 1992, *Emanuelson et al.* 1993, *Carlsson et al.* 1994). This normal range is in accordance also with the results of *Refsdal et al.* (1985) who reported a mean urea concentration of 4.6 mmol/l in the bulk milk from cows fed a ration balanced for energy and protein, and with those of *Gustafsson & Carlsson* (1993) who observed a range from 4.5 to 5.0 mmol/l in bulk milk from cows with optimal reproductive efficiency. Somewhat lower milk urea concentrations, but still within the proposed ranges, can be expected during late lactation than at peak yield *(Gustafsson et al.* 1987, *Emanuelson et al* 1993, *Carlsson et al.* 1994) and in primiparous cows than in older cows *(Oltner et al.* 1985, *Canfield et al.* 1990). Moreover,

slightly higher urea concentrations can be expected in milk samples taken by handstripping a few hours after the morning milking than in mixed samples taken from the usual morning and afternoon milkings *(Carlsson & Bergström* 1994).

It is important to point out that the normal borderline values proposed above might be somewhat adjusted depending on the kind of feedstuffs used in a ration and also depending on whether the optimum for production, health or feed costs are aspired. Specific effects of some feedstuffs are possible; it might be significant that the E_hP_l groups in the present study got hay as the only roughage and that the E_sP_h and E_sP_{hh} groups got soya meal to obtain the conditions of low protein and high protein levels, respectively. The proposed normal milk urea concentrations may also need to be adjusted if the estimated requirements of cows for energy and protein are changed. Although the official Swedish standards for milk production remain at 5.0 MJ and 40 g AAT/kg FCM, there is a tendency among advisers to increase them to 5.5 to 6.0 MJ and 43 to 45g AAT/kg FCM during the first few months of lactation and to decrease them later on. The effects of such changes on milk urea concentration are unknown and need to be investigated.

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References

Andersson G, Andersson L, Carlstrom G: Determination of milk urea by Flow Injection Analysis. J. vet. Med. A. 1986, *33,* 53-58.

Canfield RW, Sniffen CJ, Butler WR: Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 1990, *73,* 2342-2349.

Carlsson J, Bergstrom J: The diurnal variation of urea in cow's milk and how milk fat content, storage and preservation affects analysis by a flow injection technique. Acta vet. scand. 1994, *35,* 67-77.

Carlsson J , Bergstrom J, Pehrson,B· Variations with breed, age, season, yield, stage of lactation and herd in the concentration of urea in bulk milk and in individual cow's milk. Submitted for publication in Acta vet. scand. 1994.

Carlstrom G, Hallgren W, Pehrson B, Wallin O· Gas chromatographic determination of volatile fatty acids in aqueos media. Acta vet. scand. 1965, *6,* 52-58.

Church DC In: Digestive physiology and nutrition of ruminants 1976, O & B Books, Corvallis, Oregon.

Emanuelson M, Ahlin K-Å, Wiktorsson H. Long-term feeding of rapeseed meal and full-fat rapeseed of double low cultivars to dairy cows. Livest. Prod. Sci. 1993, *33,* 199-214.

Engvall A: Low milk fat syndrome in Swedish dairy cows. Thesis. Acta vet. scand. 1980, suppl. 72, 124 pp.

Gustafsson A H, Carlsson J: Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. Livest. Prod. Sci., 1993, *37,* 91-105.

Gustafsson A H, Emanuelson M, Oltner R. Wiktorsson H· Mjolkens ureahalt, dess variation och påverkan av besattning, mjölkavkastning, laktationsstadium, sasong och utfodring. (Milk urea level, its variations and how it is affected by herd, milk yield, stage of lactation, season and feeding – a field study). Report no. 165. 1987, Swedish Univ. Agric. Sci , Department of Animal Nutrition and Management, Uppsala, Sweden.

Higginbotham GE, Huber JT, Wallentine MV, Johnston NP, Andrus D. Influence of protein percentage and degradability on performance of lactating cows during moderate temperature. J. Dairy Sci. 1989, *72,* 1818-1823.

Hoffmann M, Steinhofel O· Moglichkeiten und Grenzen zur Einschatzung der Energie- und Proteinversorgung durch Kontrolle des Milchharnstoffgehaltes. (Possibilities and restrictions in using milk urea concentrations as markers of the energy and protein balance). Mh. Vet. Med. 1990, *45,* 223-227.

Karlberg B, Pacey GE: Flow injection analysis. A practical guide. Techn. Instr. Anal. Chem. 1989, *10,* 166-171.

Kirchgessner M, Kreuzer M: Milchleistung und Milchinhaltsstoffe bei Kuhen wahrend und nach Futterung überhohter Eiweissmengen. (Milk yield and milk composition in dairy cows overfed with protein). Z. Tierphysiol. Tierernähr. Futtermittelk. 1985, *54,* 99-111.

Madsen J. The basis for the proposed Nordic protein evaluation system for ruminants. The AAT-PBV system. Acta Agric. Scand.1985, Suppl. 25, pp. 9-20.

Oltner R, Emanuelson M, Wiktorsson H Urea concentrations in cow's milk in relation to milk yield, live weight, lactation number and amount and composition of feed given. Livest. Prod. Sci. 1985, *12,* 47-57.

Oltner R, Wiktorsson H: Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. Livest. Prod. Sci. 1983, *10,* 457-467.

Refsdal AO, Bævre L, Bruflot R: Urea concentration in bulk milk as an indicator of the protein supply at the herd level. Acta vet. scand.1985, *26,* 153-163.

Ropstad E, Vik-Mo L, Refsdal AO: Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta vet. scand. 1989, *30,* 199-208.

Roseler DK, Ferguson JD, Sniffen CJ, Herrema J: Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 1993, *76,* 525-534.

SAS Institute Inc.: SAS companion for the OS/2 Environment, ver. 6, Cary, NC, 1993.

Schwartz HM, Gilchrist FMC: Microbial interactions with the diet and the host animal. In: McDonald IW, Warner AC (Eds). Digestion and metabolism in the ruminant. The University of New England Publishing Unit, Armidale, Australia, 1975, pp. 165-179.

Sporndly R, Wiktorsson H: Fodertabeller till idisslare. (Feeding-tables for ruminants). Spec. skrifter 44. 1991, Sveriges lantbruksuniversitet, Uppsala, Sweden.

Volden H, Harstad OM, Henne M: Praktisk utprøving av nytt proteinvurderingssystem. (Practical test of a new protein evaluation system. Trial on agricultural schools in Norway). In: Husdyrforsøksmøtet, Ås, Norway, 1992, pp. 567-582.

Sammanfattning

Inverkan av foderstatens energi- och proteinbalans på mjolkens ureahalt. Experimentella försok bedomda medelst två olika proteinvarderingssystem.

Tjugotre mjolkkor gavs foderstater med olika mangder energi och smaltbart råprotein (DCP). När energi- och DCP-tillförseln motsvarade behovet enligt svensk norm var mjölkens ureahalt i medeltal 4,66-4,92 mmol/l (95% konfidensintervall för medeltalet). Nar mängden DCP okades utover behovet samtidigt som energitillforseln motsvarade behovet, steg mjölkureavardet ungefär i proportion till DCP-overutfodringen. Nar korna överutfodrades med energi och samtidigt underutfodrades med DCP sjönk mjolkureakoncentrationen.

Nar foderstaterna retrospektivt omräknades till AAT/PBV-systemet for proteinvärdering blev mjolkureahalten i medeltal 3,76-4,56 mmol/l (95% CI) nar foderstaten var balanserad enligt norm. Mjolkureakoncentrationen var främst beroende på foderstatens PBV-innehåll. Nar de två proteinvärderingssystemen (DCP och AAT/PBV) jämfördes forelåg en stark korrelation mellan PBV och DCP. Av ett flertal våmparametrar befanns endast ammoniakkoncentrationen vara signifikant korrelerad till mjölkureahalten.

Resultaten från detta försök och från tidigare undersökningar indikerar att en mjölkureakoncentration på 4,0-5,5 mmol/l kan betraktas som normal hos mjölkkor som bjuds en konventionell stallfoderstat.

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