Effects of different dietary energy contents on milk urea concentration in stall-fed dairy sheep

Giovanetti V., Decandia M., Boe F., Zerbini E., Cannas A., Molle G.

in

Effects of different dietary energy contents on milk urea concentration in stall-fed dairy sheep

V. Giovanetti*, M. Decandia*, F. Boe**, E. Zerbini***, A. Cannas** and G. Molle*

*Istituto Zootecnico e Caseario per la Sardegna, km 18600 SS Sassari-Fertilia, 07040 Olmedo (Italy)
**Dipart. di Scienze Zootecniche, Università degli Studi di Sassari, via Enrico de Nicola 9, 07100 Sassari (Italy)
***Cargill Srl, Regional Laboratory, via Pavia 4, 27010 Spessa (Italy)

Abstract. The objective of this study was to evaluate the effects of dietary energy content on milk urea concentration in mid and late lactating Sarda ewes, kept in metabolic crates. Forty ewes were blocked in 8 homogeneous groups, of 5 animals each, and fed with 8 pelleted diets spanning from low (i.e. fibrous diets: 1.2-1.4 Mcal of NE_L) to high (high-starch diets: 1.7-1.9 Mcal of NE_L) energy contents and with fairly similar crude protein (CP) concentration (18.4% DM on average). Based on their main ingredient, diets were denominated: corn meal (CM), wheat middlings (WM), corn flakes (CF), barley meal (BM), corn cobs (CC), beet pulp (BP), alfalfa (AA) and soybean hulls (SH). In both mid- and late-lactating sheep, milk urea concentration was affected by diet with the highest values in AA fed ewes. A negative linear relationship was found between dietary energy (NE_L, Mcal/kg DM) and milk urea (MUC, mg/100 ml) concentrations (MUC = 127.86 – 51.46 $\times$ NE_L; R^2 = 0.88, RMSE = 3.95, P < 0.001). The soluble protein fractions B_1 and, to a lesser extent, A were able to contribute in explaining MUC variations. The ratio CP/NE_L (g/Mcal) was also strictly related to MUC (MUC = –14.68 + 0.51 $\times$ $\times$ CP/NE_L; R^2 = 0.86, RMSE = 4.29, P < 0.001). This research suggests that dietary energy content can play a key role in modulating the relationship between CP or its fractions and milk urea in dairy sheep.

Keywords. Milk urea – Dietary energy – Dairy sheep.

Effet du niveau énergétique de la ration sur la concentration d'urée dans le lait de brebis alimentées à l'étable

Résumé. L'objectif de cette étude est d'évaluer les effets du niveau d'énergie sur la concentration d'urée dans le lait de brebis de race Sarde à l'auge en milieu et en fin de lactation. Quarante brebis réparties en 8 groupes homogènes ont été soumises à 8 régimes sous forme de bouchons. Les régimes étudiés avaient différents niveaux énergétiques (1,2-1,9 Mcal/kg MS de NE_L) et des niveaux azotés (MAT) assez similaires (18,4% MS en moyenne). Les régimes sont désignés selon la nature de l'ingrédient de base par : farine de maïs (CM), remoulage du blé (WM), flocons de maïs (CF), farine d'orge (BM), maïs en épis (CC), pulpe de betterave (BP), luzerne (AA) et cosses de soja (SH). La concentration d'urée dans le lait a été affectée par le régime chez les brebis en milieu ou en fin de lactation ; la concentration la plus élevée a été obtenue avec le régime AA. Une relation linéaire négative a été constatée entre la concentration énergétique de la ration (NE_L, Mcal/kg MS) et l'urée dans le lait (MUC, mg/100 ml) (MUC = 127,86 – 51,46 $\times$ NE_L ; R^2 = 0,88, RMSE = 3,95, P < 0,001). Les fractions protéiques solubles B_1 et à un moindre degré A ont contribué à expliquer les variations de MUC. Le rapport CP/NE_L (g/Mcal) a été en relation avec MUC (MUC = –14,68 + 0,51 $\times$ CP/NE_L ; R^2 = 0,86, RMSE = 4,29, P < 0,001). Les résultats suggèrent que la concentration énergétique de la ration joue un rôle clé dans la modulation des relations entre la MAT et ses fractions et l'urée du lait de la brebis.

Mots-clés. Urée dans le lait – Niveau énergétique de la ration – Brebis laitières.

I – Introduction

Milk urea concentration (MUC) has been proposed as a gauge of the protein metabolism and intake. In dairy cows several studies have shown that MUC is related to dietary CP intake, the percentage of rumen degradable protein as well as protein/energy ratio in the diet (Oltner and Wiktorsson, 1983; Butler *et al.*, 1996; Nousianen *et al.*, 2004). However, there is little information on the factors affecting MUC in lactating ewes, with particular reference to dietary energy level and the

energy source (fiber *vs* non-fiber carbohydrates). Although Cannas *et al.* (1998) have already studied the effect of dietary energy content on MUC in lactating sheep, they were able to explore only a relatively narrow range (1.55-1.65 Mcal of NE_L). Under these experimental conditions they found no effect of the dietary energy content on MUC, in contrast with the findings by other authors working on dairy cattle (e.g. Broderick, 2003). Thus, the main objective of this study was to evaluate the effect of dietary energy contents on MUC spanning from low (i.e. fibrous diets: 1.2-1.4 Mcal of NE_L) to high (high-starch diets: 1.7-1.9 Mcal of NE_L) in mid and late lactating ewes fed diets with fairly similar CP concentration (17.5-19.8% DM). Moreover the study was aimed at assessing if, despite the narrow range of dietary protein concentrations used in the experiments, CP or any protein fraction could contribute to further explain MUC variability.

II – Materials and methods

Two experiments were carried out, using mid (Experiment 1 – E1) and late lactation (Experiment 2 – E2) Sarda dairy sheep. In each experiment 40 sheep were fed 8 complete pelleted diets and had *ad libitum* access to water. Before the beginning of the experiments, the animals were fed at pasture with a mixture of the experimental pelleted diets as supplement and then confined in pen and adapted to consume only the mixture of the pelleted diets (preliminary period, 7 days in total). The ewes were then put in individual metabolic cages and submitted to the experimental feeding treatments. They stayed in the cages for 23 days in total, 14 days for adaptation (adaptation period) and 9 days for experimental measurements (experimental period). Since only 20 metabolic cages were available, the experiments were divided in two sequential trials, testing 4 diets at each time. The ewes were machine milked twice a day. The same diets were used throughout the two experiments. The ingredients and the chemical composition of the eight experimental diets are summarized in Table 1. Acronyms indicate the main ingredient of each diet: CM = corn meal; WM = wheat middlings; CF = corn flakes; BM = barley meal; CC = corn cobs; BP = beet pulps; AA = alfalfa hay; SH = soybean hulls. All diets contained dehydrated alfalfa hay as common basis. The other ingredients were added to obtain different fiber (NDF, ADF and ADL) and energy contents, with fairly similar CP concentration. In E1, 40 mid lactation sheep were blocked in 8 homogeneous groups of 5 animals each on the basis of their dags in milk (DIM) (mean ± SD, 112 ± 7), milk yield (1657 ± 153 g/d), body weight (BW) (43.6 ± 3.9 kg) and BCS (2.3 ± 0.21). The ewes were fed the pelleted diets *ad libitum*. In the first sequential trial the diets CF, BM, BP and CC were tested, in the second trial the remaining diets, CM, WM, SH and AA were fed. A different set of lactating sheep was used in the sequential trials. In E2 since the pellets of diet WM had signs of poor conservation, only seven diets were tested. The same ewes of E1, after re-randomization within the trial, were used in late lactation. The animals were blocked in 7 homogeneous groups of 5 animals each, on the basis of their DIM (mean ± SD, 200 ± 10) milk yield (1213 ± 355 g/d), BW (46.9 ± 4.2 kg) and BCS (2.6 ± 0.18). In the first sequential trial the diets CF, BM, BP and CC were tested, in the second one the diets CM, SH and AA. In order to prevent acidosis, possibly out-breaking from high concentrate intake rates during night, 10 g/d per head of sodium bicarbonate were added to the diets. In both experiments, during the first 6 days of the experimental periods, individual intake was measured. Individual milk yield was recorded three times during the experimental period, when individual milk samples were taken. Feed samples were analyzed for DM, ash, CP, EE, WSC (AOAC, 1990), NDF, ADF, ADL (van Soest *et al.*, 1991), N fractions (Licitra *et al.*, 1996) and starch (polarimetric method). The non-fiber carbohydrate (NFC) concentration was calculated as [100 – (NDF – NDIP) – CP – EE – ash]. The energy content of the diets was then calculated (NRC, 1994) using *in vivo* digestibility results (Boe, 2007). Milk analyses were performed on each sample for urea (Chem Spec 150, Bentley Instruments Inc., 4004 Peavey Road Chaska, MN 55318, USA).

A general linear model procedure was used to analyse the effect of the diet on dietary nutrients' intake and milk urea concentration within each experiment. Treatment means were separated at $P < 0.05$ thresholds using the test of Tukey. Treatment means were also used to perform the regression analysis, plotting dietary contents and intakes of nutrients against MUC and testing both the linear and the quadratic component effects of each independent variable. Quadratic components were never significant ($P < 0.05$) and hence they were discarded from the final models.

Table 1. Ingredients and chemical composition, on a DM basis, of the experimental diets

Ingredients	Diets							
	CM	**WM**	**CF**	**BM**	**CC**	**BP**	**AA**	**SH**
Barley meal				47.0				
Corn flakes			51.6					
Corn meal	67.5	9.8	8.0	1.7	5.8	1.9		5.5
Alfalfa hay	26.7	28.0	27.0	28.0	26.6	26.0	89.4	26.2
Beet pulp						40.9		
Corn cobs					32.5			
Corn germ			6.4	12.9	20.0	20.0		
Soybean hulls								62.3
Wheat middlings		56.7			10.0	6.1	5.0	
Minerals and vitamins	5.8	5.5	7.0	7.0	5.1	5.1	5.6	6.0
Chemical composition[†]								
CP	18.0	17.8	18.7	18.1	19.8	17.9	19.7	17.5
EE	3.0	3.1	2.7	3.1	4.4	3.3	2.6	2.5
Ash	7.3	8.0	8.1	9.3	8.5	8.3	11.2	9.0
NDF	23.9	26.2	28.5	33.2	45.8	44.9	45.6	51.9
ADF	12.4	13.1	14.2	16.3	22.2	21.4	29.2	36.0
ADL	2.1	2.2	2.8	3.0	3.7	3.3	6.0	2.8
NFC	51.2	47.9	47.3	41.0	26.1	31.8	25.1	23.6
WSC	3.3	4.1	3.7	3.7	3.6	4.5	4.3	3.2
Starch	35.6	34.1	28.8	25.2	12.4	8.0	7.1	3.2
NE_L E1	1.82	1.72	1.80	1.75	1.52	1.78	1.32	1.49
NE_L E2	1.76		1.88	1.69	1.53	1.73	1.20	1.44
N fractions[††]								
A	3.3	4.0	2.9	3.5	4.4	3.5	4.7	3.0
B_1	0.7	1.7	1.3	1.7	1.3	1.3	2.4	1.4
B_2	10.6	9.1	9.1	8.2	9.5	6.8	8.4	8.6
B_3	1.9	2.0	3.4	3.2	2.9	4.4	2.7	2.6
C	1.5	1.0	1.9	1.5	1.7	1.8	1.5	1.9

[†]CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; NFC: non-fibre carbohydrates; WSC: water soluble carbohydrates.
[††]N fractions: A = NPN; B_1 = buffer soluble true protein; B_2 = (buffer insoluble protein – neutral detergent soluble protein); B_3 = (neutral detergent insoluble protein – acid detergent insoluble protein); C = acid detergent insoluble protein (Licitra *et al.*, 1996).

III – Results and discussion

Experimental diets affected the intake of nutrients (Table 2). Fibrous diets (CC, AA, BP, SH) showed higher DM, CP and NDF intake than high NFC diets (CM, WM, CF, BM), suggesting that intake was regulated by energy demand and was not limited by the fibre content of the diets, because of the small particle size of the NDF of the pellets (Boe, 2007). MUC was affected by the diet in both experiments (Table 3). Higher values were found in the low (AA, CC and SH) than in the high (CM, WM, CF, BM) NFC diets. In particular, the highest MUC values were found in the ewes fed the AA diet, which had also high CP/NE_L (E1 = 149; E2 = 164 g/Mcal/kg DM). The sheep fed the BM diet, with high energy content, showed in late lactation high MUC values, probably because of the slightly higher CP intake (Table 2). The lowest value of MUC was detected with the CM diet (Table 3). During late lactation, the typical mating period for Sarda sheep, MUC of several dietary groups (Table 3) was either close to or higher than the thresholds of 45 mg/dl (bulk milk samples) or 55 mg/dl of (individual milk samples) above which a marked reduction in fertility of artificially inseminated Sarda sheep is expected to occur (Branca *et al.*, 2000). Pooling the dietary

treatment means of each of the two experiments, a negative linear relationship was found between dietary energy (NE$_L$, Mcal/kg DM) and milk urea (MUC, mg/100 ml) concentrations (Table 4). This relationship could be explained by the positive effect of dietary non-fiber carbohydrates on microbial protein synthesis with consequent reduction of ammonia concentration in the rumen.

Table 2. Intake of nutrients by ewes in mid (E1) and late lactation (E2) fed the experimental diets

		Diets								P <
		CM	WM	CF	BM	CC	BP	AA	SH	
DMI (g/d)	E1	1718[c]	1864[bc]	1871[abc]	1849[c]	2602[ab]	2055[abc]	2196[abc]	2634[a]	0.01
	E2	890[c]		1295[bc]	1752[ab]	2182[a]	1992[a]	1903[ab]	1604[ab]	0.01
CPI (g/d)	E1	310[c]	332[bc]	349[bc]	334[bc]	516[a]	367[bc]	432[abc]	462[ab]	0.01
	E2	160[d]		242[cd]	317[bc]	433[a]	356[ab]	375[ab]	281[bc]	0.01
NDFI (g/d)	E1	410[c]	489[c]	532[c]	614[c]	1191[ab]	924[b]	1002[b]	1367[a]	0.01
	E2	213[c]		369[bc]	582[b]	999[a]	895[a]	869[a]	832[a]	0.01
NFCI (g/d)	E1	881[ab]	908[a]	891[a]	758[abc]	708[abc]	652[abc]	559[c]	609[bc]	0.01
	E2	461[bc]		612[ab]	724[a]	594[abc]	634[ab]	480[bc]	375[c]	0.01
NE$_L$I (Mcal/d)	E1	3.13	3.22	3.37	3.23	3.96	3.65	2.91	3.93	0.09
	E2	1.56[d]		2.41[bcd]	2.97[abc]	3.34[ab]	3.46[a]	2.27[cd]	2.28[cd]	0.01

[a,b,c,d]Values within experiment with different superscript letters differ at P < 0.05.

Table 3. Milk urea concentration of ewes in mid (E1) and late lactation (E2) fed the experimental diets

		Diets								P <
		CM	WM	CF	BM	CC	BP	AA	SH	
Milk urea (mg/dl)	E1	33.36[c]	38.71[bc]	33.52[c]	36.25[bc]	48.37[ab]	34.67[bc]	57.16[a]	43.51[abc]	0.01
	E2	37.72[c]		38.39[c]	46.15[bc]	44.91[bc]	38.81[c]	72.79[a]	56.18[b]	0.01

[a,b,c]Values within experiment with different superscript letters differ at P < 0.05.

Table 4. Predictions of milk urea concentration (dependent variable, mg/100 ml) according to linear fixed effects regression models based on dietary nutrients as independent variables (model: Y = a + + bX, where a is the intercept and b represents the regression slope). Based on treatment means

Independent variable	N	a	SE	P<	b	SE	P<	RMSE	CV	R^2	P<
CP	15	−73.0	56.1	0.22	6.3	3.0	0.057	9.81	22.3	0.25	0.057
A	15	5.1	14.1	0.72	10.7	3.8	0.015	8.95	20.3	0.38	0.015
B$_1$	15	19.9	7.0	0.014	16.5	4.6	0.003	8.00	18.2	0.50	0.003
(A+B$_1$)	15	2.3	10.9	0.83	8.1	2.1	0.002	7.72	17.5	0.54	0.002
NDF	15	20.7	9.5	0.048	0.6	0.2	0.025	9.28	21.1	0.33	0.025
NFC	15	67.7	7.7	0.001	-0.7	0.2	0.007	8.47	19.2	0.44	0.007
NE$_L$	15	127.8	8.7	0.001	-51.5	5.3	0.001	3.95	9.0	0.88	0.001
CP/NE$_L$	15	−14.68	6.75	0.049	5.08	0.57	0.001	4.29	9.74	0.86	0.001

This is also confirmed by the negative relationship between NFC content and MUC (Table 4). The coefficient of determination of dietary CP concentration regressed against MUC was low (R^2 = 0.25, P < 0.06), probably for the limited range of CP concentration. The strength of the relationship was

improved when MUC was related with the dietary B_1 protein fraction ($R^2 = 0.50$, $P < 0.01$) and with the total amount of soluble dietary N ($A + B_1$) ($R^2 = 0.54$, $P < 0.01$; Table 4). Energy content of diet and N soluble fractions were then the main explanatory variables for MUC. These findings overall suggest that the concentration of urea in this study depended more upon the amount of protein fermented in the rumen than that absorbed in the intestine. Unfortunately, N fractions have moderate to low reproducibility and repeatability (Bovera *et al.*, 2003) and this can be regarded as a limit for their practical use as nutritional index, compared with CP.

The CP/NE_L (g/Mcal) ratio was also strictly related to MUC as shown in Table 4. Concerning the linear relationships between MUC and intake components, only NFC intake was significantly related with milk urea, even if with low determination coefficient ($R^2 = 0.38$, $P < 0.05$).

IV – Conclusions

In this study, a negative linear relationship was found between dietary energy content and MUC in lactating sheep. Urea concentration in milk was never strongly related with dietary CP content, probably because of its small range of variation. In contrast, in the specific experimental conditions of this study, the soluble protein fractions B_1 and to a lesser extent, A, were able to contribute in explaining MUC variations. Overall the CP/NE_L ratio proved to be the best singular predictor of MUC. From the practical standpoint, this research confirms that MUC can be regarded as useful predictors of protein nutrition in dairy sheep. It also suggests that dietary energy content plays a pivotal role in modulating the relationship between MUC and dietary CP. Further research is warranted for improving the use of this indicator, particularly for grazing sheep.

Acknowledgements

References

AOAC, 1990. *Official Methods of Analysis*, 15th edn. Arlington, VA, USA: Association of Official Analytical Chemists.

Boe F., 2007. Effects of dietary carbohydrates on milk production, feed utilization, and feeding behaviour of dairy sheep. PhD Thesis: University of Sassari. 191 p.

Bovera F., Spanghero M., Galassi G., Masoero F. and Boccioni A., 2003. Repeatability and reproducibility of the Cornell Net Carbohydrate and Protein System analytical determinations. In: *Ital. J. Anim. Sci.*, 2. p. 41-50.

Branca A., Molle G., Sitzia M., Decandia M. and Landau S., 2000. Short-term dietary effects on reproductive wastage after induced ovulation and artificial insemination in primiparous lactating Sarda ewes. In: *Animal Rep. Sci.*, 58. p. 59-71.

Broderick G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. In: *J. Dairy Sci.*, 86. p. 1370-1381.

Butler W.R., Calaman J.J. and Beam S.W., 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. In: *J. Anim. Sci.*, 74. p. 858-865.

Cannas A., Pes A., Mancuso R., Vodret B. and Nudda A., 1998. Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. In: *J. Dairy Sci.*, 81. p. 499-508.

Licitra G., Hernández T.M. and van Soest P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. In: *Anim. Feed Sci. Technol.*, 57. p. 347-358.

Nousianen J., Shingfield K.J. and Huhtanen P., 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. In: *J. Dairy Sci.*, 87. p. 386-398.

NRC, 1994. *Nutrient Requirements of Sheep*. Washington DC, USA: NRC.

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

van Soest P.J., Robertson J.B. and Lewis B.A., 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. In: *J. Dairy Sci.*, 74. p. 3583-3597.