

THE EFFECT OF ENERGY AND PROTEIN LEVELS OF FEEDING ON MILK YIELD AND RUMEN FERMENTATION IN DAIRY EWES

Mierlita Daniel

University of Oradea; Environmental Protection Faculty, Gen. Magheru Street, no. 26, Oradea, e-mail:
dadi.mierlita@personal.ro

Abstract

Keywords: energy and protein in the diet, milk, rumen fermentation, dairy ewes.

The objective of this trial was to evaluate the effect with different level energy and protein on diet of characteristics fermentation ruminal, yield and quality of sheep milk. Consequently, experimental diets were constituted by a 2 x 2 factorial arrangement of energy level in diets (Low – 0,90 UFL/kg DM vs. High – 0,97 UFL/kg DM) and protein level (Low – 14% CP vs. High – 16% CP). On a dry matter (DM) basis, forage : concentrate ratios were 73 : 27 (low energy diets) and 57 : 43 (high energy diets). Protein level diet were given either with soybean meal (5,5% of DM) or without. Increased levels of protein and energy of diet in lactating sheep, influenced positively the quantitative and qualitative production of milk. So, the average daily production of milk increased up to 14.7% ($p \leq 0.05$) and, also, fat, protein and lactose content, but it decreased the casein content (which could reduce the processing performance of milk in cheese) and nonproteinic nitrogen.

After feeding, ewes fed the high energy and protein diets had higher proportion of propionate and lower proportion of acetate and acetate/propionate ratio than those fed the low energy and protein diets. Sheep fed the high energy and high protein diets had lower $\text{NH}_3\text{-N}$ in ruminal fluid, than those fed the low diets ($p \leq 0,05$).

INTRODUCTION

The type and proportion of forages and concentrates in the diet may also have a significant effect on the yield and composition of the milk given by dairy ewes (Kukuk et al., 2001; Mele et al., 2006; Cannas et al., 1998; Guitard et al., 1996; Bocouier et al. 2001; Bencini et al., 1997). The concentration of fat in the milk is correlated positively with the concentration of fibre in the diet. Bencini et al. (1997) have calculated the relationship between milk fat and NDF in the diet ($\text{fat}\% = 4,59 + 0,05 \text{ NDF}$; $r = 0,48$).

The protein content of the diet affects the quantity and the partition of nitrogenous substances in the milk; that milk protein was significantly reduced if ewes were fed a protein deficient diet. Milk yield and concentration of milk fat can be increased by increasing the protein content of the diet (Bencini et al. 1997).

The objective of this trial was to evaluate the effect of energy and protein content in the effect with different level energy and protein on diet of characteristics fermentation ruminal, yield and quality of sheep milk.

MATERIAL AND METHODS

Animals and diets

Researches were conducted on 48 lactating Țurcană sheep (with a live weight of $46,4 \pm 4,2$ kg and milk yield $0,61 \pm 0,12$ kg), being at lactation 3 and 4, respectively, months 2-4 of lactation. The sheep were divided into 4 groups of 12 sheep/lot in a preexperimental period of 14 days, when feeding was made with a basic portion and it was followed the average milk yield per lot, so it does not differ significantly from a lot to another. The animals were kept in collective boxes (one box for a lot) on the permanent straw bedding, and food was administered twice daily (at 7.00 and 18.00), ad libitum.

The analytical composition of the diet (table 1) have also been reported. The 4 experimental diets differed in terms of level energy and protein, of forage/concentrate ratio respectively (diet composition expressed as the percentage of dry matter):

- A diet with low energy and low protein (forage/concentrate ratio – 73 : 27), made up of forage (73%), corn grein (18,8%) and triticale meal (7,2%);
- A diet with low energy and high protein (forage/concentrate ratio – 73 : 27), made up of forage (73%), corn grein (13,3%), triticale meal (7,2%) and soybean meal (5,5%);
- A diet with high energy and low protein (forage/concentrate ratio – 57 : 43), made up of forage (57%), corn grein (32,6%) and triticale meal (9,4%);
- A diet with high energy and high protein (forage/concentrate ratio – 57 : 43), made up of forage (57%), corn grein (29,2%), triticale meal (7,3%) and soybean meal (5,5%).

Table 1

Feed ingredients of the experimental diets of dairy ewes (% DM)

<i>Energy</i> level	Low – 0,90 UFL		High – 0,97 UFL	
	14% CP	16% CP	14% CP	16% CP
<i>Protein</i> level				
Grass hay	13,8	13,8	8,9	8,9
Mixed grass pasture	28,6	28,6	18,5	18,5
Alfalfa grass	30,7	30,7	29,8	29,8
Corn grein	18,8	13,3	32,6	29,2
Triticale meal	7,2	7,2	9,4	7,3
Soybean meal	-	5,5	-	5,5
Minerals and vitamins ¹	1,0	1,0	1,0	1,0
<i>Forage : concentrate</i>	<i>73 : 27</i>	<i>73 : 27</i>	<i>57 : 43</i>	<i>57 : 43</i>

1 : Contains (g/kg): I, 1,22; Mn, 103; Zn, 110; Fe, 137; Cu, 16; Co, 0,35; Se, 0,31; (IU/kg): vitamin A, 11000; vitamin D, 3200; vitamin E, 56.

Forage (grass hay, mixed grass pasture and lucerne grass) was fed separately twice a day, controlling the actual intakes of both forage and concentrate.

The chemical composition of the diet was determined according to AOAC methods (AOAC, 1990). Fiber fractions (NDF and ADF) were analyzed according to the method described by Van Soest et al. (1991). Non structural carbohydrates (NSC) were calculated according to Van Soest et al. (1991). Net energy for lactation (NE_L) and intestinal digestible protein (PDIN and PDIE) were estimated from INRA tables (1989).

Experimental design

Experimental diets were constituted by a 2 x 2 factorial arrangement of level energy in diets (Low – 0,90 UFL/kg DM vs. High – 0,97 UFL/kg DM) and level protein (Low – 14% CP vs. High – 16% CP). After a 10-day adaptation period to the diets experiments, meant to let the rumen microbes get accustomed to the changed diet.

Milk yield and rumen fermentation

The animals were milked twice daily (at 06:00 h and 18:00 h). The two samples were gathered in a single sample according to the morning and afternoon yield. Milk samples were then analyzed to determine fat matter (Gerber method), total nitrogen (Kjeldahl method, N x 6,38 = total protein), lactose (infrared method, Combifoss 4000 FOSS, Hillerod, Denmark), and fatty acids composition.

On week 6 experiments, ruminal fluid was collected before and 2 h after the morning feeding. Following immediate determination of pH, ruminal fluid was acidified with 25% (wt/vol.) H₃PO₄ and frazen until analysis of ammonia (Wheatherburn, 1967) and VFA (Kristensen et al. 2000).

Statistical analysis

Ruminal samples obtained before and 2 h after feeding were analyzed separately with a model including the fixed effects of energy level, protein level diet and their interaction. All other data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., 1999). The statistical model included energy level, protein level, energy x protein interaction and residual error. Data are reported as least squares means \pm SEM. Overall differences between treatment means and interaction for level of energy level and protein level were considered to be significant when $p \leq 0,05$.

RESULTS AND DISCUSSION

Diet composition

Portions with high energy value (0.97 UFL / kg DM) are characterized by a lower content of fibers (NDF and ADF) and a higher content of ether extract (EE) and nonstructural carbohydrates (NSC), comparing to low energy value portions (table 2). The increase of food protein level from 14% CP to 16% CP, by introducing soybean meal (5.5% of DM), regardless of energy level, it determines the increase of ether extract (EE) share and the decrease of non-structural carbohydrates (NSC) proportion.

Table 2

Chemical composition and nutritional values of experimental diets (% DM)¹

Energy level	Low – 0,90 UFL		High – 0,97 UFL	
	14% CP	16% CP	14% CP	16% CP
CP (crude protein)	14,18	16,10	13,87	16,17
NDF	44,10	44,0	35,4	35,1
ADF	26,90	26,60	24,70	24,40
NSC	16,4	14,2	34,6	31,4
EE	1,72	2,17	2,52	3,08
PDIE (g/kg DM) ²	90,1	98,6	94,3	112,8
PDIN (g/kg DM) ²	86,1	102,4	89,3	108,7
NE _i (kcal/kg DM) ³	1547	1561	1664	1678

¹ Data presented are least square means (n = 4 samples per diet). ²PDIN and PDIE = digestible CP in the intestine from microbial protein synthesis when availability of fermentable N in the rumen is limiting, and from microbial protein synthesis when availability of energy in the rumen is limiting, respectively (INRA, 1989). ³ Calculated values (INRA, 1989).

DM intake, milk production and composition

Average dry matter intake (DMI) did not differ between experimental groups (table 4).

Increasing energy level by increasing the proportion of concentrates from ration structure led to a significant increase of NEL intake (kcal / kg) ($p \leq 0.01$), but also to the quantitative and qualitative improvement of milk production. It was increased the average daily production of milk ($p \leq 0.05$) and its fat, protein and lactose content and it was reduced the content in nitrogen and non protein casein, which could negatively influence the performance of processing milk into cheese (table 3).

Table 3

The effect of energy and protein levels of feeding on production and composition of sheep milk¹.

Energy level	Low – 0,90 UFL		High – 0,97 UFL		SEM	p values of effects ²		
	14% CP	16% CP	14% CP	16% CP		E	CP	ExCP
DMI (kg/day)	1,828	1,837	1,890	1,885	0,08	NS	NS	NS
NE _L intake (kcal/day)	2828	2867	3145	3163	174	**	NS	NS
Milk yield (kg/day)	66,7	66,5	64,7	67,3	0,82	*	*	NS
Milk fat (g/l)	53,4	54,6	58,2	57,6	0,41	*	NS	NS
Milk protein (g/l)	47,4	46,8	48,8	48,3	0,40	*	NS	NS
Milk lactose (g/l)	40,4	38,6	38,3	37,6	0,44	**	NS	NS
Casein (g/l)	39,0	44,6	40,6	43,3	2,61	*	**	**
Fat yield (g/day)	31,2	36,6	36,5	37,0	1,02	*	NS	NS
Protein yield (g/day)	27,7	31,40	30,6	31,0	0,74	**	NS	NS
Lactose yield (g/day)	1,34	1,86	1,17	1,40	0,06	**	**	***
Non proteic nitrogen	75,6	73,82	72,8	68,7	0,91	**	NS	*
Casein, % of CP								

¹ Data presented are least square means – n = 12 ewes per group.

² E = effect of energy level, CP = effect of protein level, ExCP = interaction between energy and protein level diet. *** : $p \leq 0,001$; ** : $p \leq 0,01$; * : $p \leq 0,05$.

Protein level of feed influenced positively the production of milk ($p \leq 0.01$) and the content in fat of milk ($p \leq 0.05$), but increased milk content in non-proteic nitrogen ($p \leq 0.01$).

Robinson et al.; Calderon-Cortes et al.; Cowan et al. (cit. Bencini R. 1997), Pulina et al. (1995), Bocquier et al. (2001), Gargouri, (2005); Schmidely et al. (2001), all have shown that milk yield and concentration of milk fat can be increased by increasing the protein content of the diet. By contrast Sinclair et al. (1990), Lynh et al. (1991), Rossi et al. (1991) and Cannas et al. (1995) reported that high concentrations of protein in the diet can increase the concentration of non proteic nitrogen and especially urea

which results in a poorer processing performance of the milk. Feeding high-energy diets generally induce slow milk fat content in cow, ewes and goats (Bauman et al., 2001; Sauvant et al., 2000). Positive effect of feeding high-energy combined with high-protein diet on milk fat/protein ratio was also obtained because milk protein content and protein yield ewes not reduced, in agreement with previous observations in sheep and goats (Chilliard et al., 2003; Schmidely et al., 2005).

Rumen fermentation

Ruminal pH, ruminal ammonia-N and VFA (acetate, propionate and acetate/propionate ratio) were affected by the dietary factors or by their interaction (table 4). Sheep fed the high energy diets to have lower acetate and higher butyrate molar proportion before feeding than those fed the low energy diets. After feeding, ewes fed the high energy and protein diets had higher proportion of propionate and lower proportion of acetate and acetate/propionate ratio than those fed the low energy and protein diets. Sheep fed the high energy and high protein diets had lower NH₃-N in ruminal fluid, than those fed the low diets ($p \leq 0,05$).

Table 4

Fermentation characteristics in the ruminal fluid collected of 2 h after feeding in dairy ewes fed low - or high – energy and protein diets¹.

Energy level	Low – 0,90 UFL		High – 0,97 UFL		SEM	p values of effects ²		
	14% CP	16% CP	14% CP	16% CP		E	CP	ExCP
pH	6,70	6,82	6,30	6,21	0,05	*	NS	NS
VFA, mol/100 mol								
- Acetate	68,4	67,5	64,0	65,3	0,50	***	NS	*
- Propionate	16,7	18,2	19,0	21,0	0,47	***	*	NS
- Butyrate	12,2	11,9	13,3	11,4	0,31	NS	*	NS
- Isobutyrate	1,82	1,40	2,31	1,34	0,07	NS	*	NS
- Valerate	0,77	0,90	0,96	0,87	0,03	NS	NS	*
- Isovalerate	0,11	0,10	0,43	0,09	0,05	NS	NS	*
Acetate/propionate	4,09	3,71	3,36	3,11	0,11	***	*	NS
Ammonia – N, mM	3,38	4,30	3,13	3,01	0,45	NS	NS	*

¹ Data presented are least square means – n = 4 ewes per group – 2 h after feeding

² E = effect of energy level, CP = effect of protein level, ExCP = interaction between energy and protein level diet. *** : $p \leq 0,001$; ** : $p \leq 0,01$; * : $p \leq 0,05$.

CONCLUSIONS

1. Increased energy level of lactating sheep food(0.90 UFL / kg DM vs. 0.97 UFL / kg DM) by increasing the share of concentrates in the ration (from 27% to 43% in DM) and protein level (from 14% CP to 16% CP of DM) by introducing soybean groats (5.5% of DM), positively influenced the quantitative and qualitative production of milk and, in particular, the profile of fatty acids. This way, the average daily production of milk increased up to 14.7% ($p \leq 0.05$) and, also, fat content, protein and lactose content increased, but decreased the casein content (which could reduce the performance of processing milk in cheese) and non protein nitrogen.
2. Sheep fed the high energy diets to have lower acetate and higher butyrate molar proportion before feeding than those fed the low energy diets. After feeding, ewes fed the high energy and protein diets had higher proportion of propionate and lower proportion of acetate and acetate/propionate ratio than those fed the low energy and protein diets. Sheep fed the high energy and high protein diets had lower $\text{NH}_3\text{-N}$ in ruminal fluid, than those fed the low diets ($p \leq 0,05$).

Acknowledgments

Financial support for this research the Ministry of Education and Research, Romania, is gratefully acknowledged – CNCSIS, PN II project, ID_679, no. 1082/2008.

REFERENCES

1. Addis M., A. Cabiddu, G. Pinna, M. Decandia, G. Piredda, A. Pirisi and G. Molle (2005) – Milk and cheese fatty acid composition in sheep fed mediterranean forages with reference to conjugated linoleic acid cis-9, trans-11. *J. Dairy Sci.* 88: 3443-3454.
2. Andrade, P.V.D., Schmidely, Ph., (2006) – Influence of percentage of concentrate in combination with rolled canola seed on performance, rumen fermentation and milk fatty acid composition in dairy goats. *Livestock Science*.
3. AOAC, (1990) – Official Methods of Analysis of the AOAC. 15th ed., Association of Official Analytical Chemists, Arlington, VA, USA.
4. Bocquier F., G., Caja (2001) – Production et composition du lait de brebis: effets de l'alimentation. *INRA Prod. Anim.*, 14 (2): 129 – 140.

5. Chilliard, Y., A. Ferlay, J. Rouel, and G. Lamberet (2003) – A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. *J. Dairy Sci.* 86: 1751 – 1770.
6. Chin, S. F., W. Liu, J. Storkson, Y. L. Ha, M. V. Pariza (1992) – Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogenes. *J. Food Comp. Anal.* 5: 185 – 197.
7. Dhiman, T., G. R. Anand, L.D. Satter, and M.W. Pariza (1999) – Conjugated linoleic acid content of milk cows fed different diets. *J. Dairy Sci.* 82: 2146 – 2156.
8. Gargouri A., (2005) – Production et composition du lait de brebis: effets de l'apport de lipides proteges. *Rev. Elev. Med. vet.*, 58 (3): 183 – 190.
9. INRA, (1989) – Ruminant nutrition. Recommended allowances and feed tables. Institut National de la Recherche Agronomique ed., Paris, France.
10. Kukuk O., Hess B.W., Ludden P.A., Rule D.C. (2001) – Effect of forage/concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. *J. Anim. Sci.* 79: 2233 – 2240.
11. Martini M., C. Scolozzi, D., Gatta, F., Taccini, P., Verita, (2004) – Effects of olive oil calcium soaps and phase of lactation on the fatty acid composition in the milk of Massese ewes. *Ital. J. Anim. Sci.*, 3: 353 – 362.
12. Mele M., A. Buccioni, F. Petacchi, A. Serra, S. Banni, M. Antongiovanni, P. Secchiari (2006) – Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. *Anim. Res.* 55: 273 – 285.
13. Mele M., A. Serra, A. Buccioni, G. Conte, A. Pollicardo, P. Secchiari (2008) – Effect of soybean oil supplementation on milk fatty acid composition from Saanen goats fed diets with different forage:concentrate ratios. *Ital. J. Anim. Sci.*, vol. 7, 297 – 311.
14. Ney D.M., (1991) – Symposium: the role of the nutritional and health benefits in the marketing of dairy products. *J. Dairy Sci.* 74: 4002 – 4012.
15. SAS, (1999) – Users Guide: Statistics. SAS Institute Inc., Cary, NC, USA.
16. Schmidely P., D., Sauvant (2001) – Taux butyreux et composition de la matiere grasse du lait chez las petits ruminants: effets de l'apport de matieres grasses ou d'aliment concentre. *INRA Prod. Anim.*, 14 (5): 337 – 354.
17. Secchiari P., Mele, M., Serra, A., Andreotti L. (2001) – Conjugated linoleic acid (CLA) content in milk of three dairy sheep breeds. *Prog. Nutr.* 3 (4): 37 – 42.
18. Strata, A., (2000) – Alimentazione e salute. Scenario attuale e prospettive future. *Prog. Nutr.* 2 (1): 3-11.
19. Van Soest, P.J., Robertson, J.B., Lewis, B.A., (1991) – Methods for ditary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583 – 3597.