Journal of Animal and Feed Sciences, 19, 2010, 211–217

Effect of a protein level in the diet on fatty acid profile in goat milk*

M. Czauderna[1], J. Kowalczyk and J.P. Michalski

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences
05-110 Jabłonna, Poland

(Received 19 November 2009; revised version 17 March 2010; accepted 6 May 2010)

ABSTRACT

The aim of the study was to evaluate the influence of feeding goats diets with different protein levels (11.4, 13.3 and 16.9%) and a small change of fatty acid contents on the concentrations of *trans11*C18:1 and conjugated linoleic acid (CLA) isomers in milk. The experimental design was 3 × 3 Latin Square. Each 22-day experimental period consisted of 14 days adaptation to the diets and 8 days for milk samples collection. Separation of methylated CLA isomers was achieved using GLC. Milk production was nearly the same when the low- and medium-protein diets were fed, while the high-protein diet resulted in a tendency to increase milk production. The concentrations of atherogenic (A-SFA), thrombogenic (T-SFA) and total saturated fatty acids (SFA) were similar in milk from goats fed the diets containing low- and medium-protein contents, whereas the diet containing the highest protein content resulted in an increase in the daily production of SFA and A-SFA in milk. The concentration and daily production of *cis9trans11*CLA, *trans10cis12*CLA, the sum of CLA isomers and usually *trans11*C18:1 and the sum of *trans,trans*CLA isomers in milk increased as the dietary protein level increased. The higher dietary protein content resulted in the decrease in the concentration ratio of SFA and unsaturated fatty acids (UFA) (SFA/UFA) and tended to the increase in the capacity Δ9-desaturation.

KEY WORDS: dietary protein, conjugated linoleic acid isomers, fatty acids, milk, goats

* Supported in part by the State Committee for Scientific Research, Grant No. 2 P06Z 06329
[1] Corresponding author: e-mail: m.czauderna@ifzz.pan.pl

INTRODUCTION

Milk and dairy products make a significant contribution to human nutrition, including numerous vitamins and minerals, conjugated linoleic acid (CLA) isomers and high-quality proteins. Although milk and dairy products provide essential nutrients, there is growing demand to increase the content of health-promoting components in milk and dairy products (Andrade and Schmidely, 2006; Nudda et al., 2006; Czauderna et al., 2007b). Decreasing the milk concentration of atherogenic (A-SFA), thrombogenic (T-SFA) fatty acids through dietary manipulations has gained significant attention because of its implications for human health (Ulbricht and Southgate, 1991). Dietary manipulations should result in increasing the concentration of n-3 polyunsaturated fatty acids (n-3PUFA), *cis11*C18:1 (*c11*C18:1), *trans11*C18:1 (*t11*C18:1), CLA isomers and the value of the n-3PUFA/n-6PUFA ratio (Chilliard et al., 2003; Leiber et al., 2005; Rioux et al., 2005). Differences between goats and cows in milk fat content and fatty acid (FA) profile in response to dietary manipulations were recently reviewed (Chilliard and Ferlay, 2004; Chichlowski et al., 2005; Andrade and Schmidely, 2006). Indeed, goat milk is reported to contain more of the essential fatty acids, linoleic and arachnodonic acids, in addition to a higher proportion of short-chain and medium-chain fatty acids. Moreover, goat's milk may also have advantages when it comes to allergies. Goat's milk contains only trace amounts of an allergenic casein protein, alpha-S1, found in larger concentration in cow's milk. Goat's milk is also a very good source of calcium, magnesium, potassium, iron and tryptophan, as well as is also reach in phosphorus, riboflavin (vitamin B_2) and valuable proteins (e.g., the casein family of protein, the serum (whey) proteins). Milk proteins contain all 9 essential amino acids required by humans.

To our knowledge, there are few studies on the effect of dietary manipulation on the concentration of unsaturated fatty acids (UFA) and especially the CLA isomer profile in goats' milk (Czauderna et al., 2007b). Therefore, the objective of current investigation was to evaluate the effect of feeding different protein levels in the diet with minute changes of fatty acid contents on the concentrations of precursors of CLA isomers, CLA isomers, long-chain PUFA (LPUFA) and especially n-3LPUFA in milk of goats.

MATERIAL AND METHODS

The experiment was performed on 3 dairy primiparous Alpine goats (~12 months old) in similar lactation phases. The animals were housed and handled in accordance with protocols approved by the Local Animal Care

and Use Committee (The Agricultural University of Warsaw, Poland). The experimental design was 3 × 3 Latin squares. Goats were housed in separate metabolic cages, fed every 6 h with three diets containing 11.4, 13.3 and 16.9% of crude protein in DM. Increasing the content of protein in the diets resulted also in slightly increasing the fatty acid content (Table 1). The 22-day period consisted of 14 days adaptation to the diets and 8 days for milk collection; water

Table 1. Ingredient and chemical composition of diets, diet intake and milk production

Item	Diet, protein level		
	low	medium	high
Diet intake, kg/day/goat	1.65	1.62	1.78
dry mater (DM)	1.47	1.44	1.59
crude protein	0.168	0.191	0.273
metabolizable energy[1], MJ/day	17.5	17.0	18.7
Diet ingredients:			
hay			
DM, g/kg hay		915	
crude protein, g/kg DM		113	
crude fibre, g/kg DM		307	
metabolizable energy, ME		10.6 MJ/kg	
concentrate, g/kg			
barley grain	116	115	116
dried beet pulp	401	400	404
wheat-starch	359	288	136
soyabean meal	104	177	324
PolfamixOK	20	20	20
total fatty acids	3.20	3.78	5.49
ME, MJ/kg concentrate	14.1	12.6	12.4
DM, g/kg	892	897	897
crude protein, g/kg DM	111	151	216
crude fibre, g/kg DM	85	84	81
Milk production, kg/day/goat[2]	1.57[a]	1.50[a]	1.78[b]
Milk fat, %	2.72[a]	2.78[ab]	2.94[b]
Milk total nitrogen, %	0.47[a]	0.49[ab]	0.50[b]

[1] metabolizable energy calculated according to Energy Allowances and Feeding System for Ruminants, MAFF, London Tech. Bull., p. 33;

[2] [a,b] - values sharing different letters differed at $P<0.05$

was freely available. Goats were milked twice a day at 06.00 and 18.00, milk was weighed and pooled for 24 h. Milk samples were stored at -20°C until analysis. Thawed milk samples were warmed to 38°C and sonicated for 1 min. Milk saponification, fatty acid (FA) extraction, FA methylation followed by separations of methylated FA using the GLC-FID method as previously described (Czauderna et al., 2007a).

The atherogenic SFA index (A_{index}) was calculated according to the equation (1) (Ulbricht and Southgate, 1991):

$$A_{index} = (C12:0+4*C14:0 +C16:0)/(MUFA + FAn-6 + FAn-3) \quad (1)$$

The thrombogenic SFA index (T_{index}) was calculated according to the equation (2) (Ulbricht and Southgate, 1991):

$$T_{index} = (C14:0 + C16:0 + C18:0)/0.5*MUFA + 0.5*FAn-6 + 3*FAn-3 + FAn-3/FAn-6) \quad (2)$$

Statistical analyses of the effects of diets containing different protein levels were conducted using the non-parametric Mann-Whitney U test. The Statistica v. 6 package was applied (Statistica by StatSoft, 2002. Web:www.statsoft.pl).

RESULTS AND DISCUSSION

The data summarized in Table 1 showed that there were substantial differences between groups in crude protein intake. Milk production was practically the same when the low- and medium-protein diets were fed, whereas the high protein diet resulted in a tendency ($P<0.083$) to increase milk production in comparison with animals fed the low-protein diet.

The obtained results (Table 2) documented that various protein levels in the diet resulted in inconsistently changes in daily production of C16:0 in milk, whereas the increase of the concentration of protein in the diet reduced the concentration of C16:0 in milk. Indeed, feeding the diet containing the lowest content of protein resulted in the increase in the concentration of C16:0 in milk, while the highest content of protein in the diet most efficiently decreased the concentration of C16:0 in milk ($P<0.05$). As C16:0 to be particularly linked with the incidence of coronary heart disease (CHD), these results constitute valuable information for nutritionists in the context of carrying out research to improve the nutritional quality of food for humans and domestic animals.

We decided to analyse whether the protein level in the diet may affect the milk atherogenic SFA concentration and interaction of some fatty acids that may have atherogenic and thrombogenic properties. The results of our studies confirmed that the concentration of protein in the goat diet negatively correlated with the values of the atherogenic and thrombogenic indexes (i.e. A_{index} and T_{index}) in milk. Therefore, we argue that increasing the protein concentration in the diet resulted in improving the nutritional quality of milk due to decrease in the values of A_{index} and T_{index} $A_{i,}$ particularly A_{index}.

Furthermore, as the dietary protein level increased (from 11.4 to 13.3% or to 16.9%), the concentration and daily production of *c11*C18:1 (the precursor of *c9c11*CLA) in milk was elevated ($P<0.05$), although the diet containing the

Table 2. Fatty acid profile in milk of goats fed diets containing various protein concentrations[1]

Fatty acid content in milk	Diet, protein level						Correlation[14]	
	low		medium		high			
	mg/ml	g[2]	mg/ml	g[2]	mg/ml	g[2]	$r_{mg/ml}$	r_g
C16:0	2.35[a]	3.77[a]	2.12[ab]	3.48[a]	2.11[b]	3.80[a]	0.486	0.259
A_{index}[3]	2.45[Aa]		1.99[b]		1.84[Bb]		-0.894	
T_{index}[4]	5.00[a]		4.25[ab]		3.99[b]		-0.900	
*c11*C18:1	0.056[a]	0.091[a]	0.070[b]	0.111[bc]	0.065[b]	0.118[c]	0.276	0.901
Σ(*c11+t11*)[5]	0.12[a]	0.19[a]	0.16[b]	0.25[b]	0.17[c]	0.30[c]	0.873	0.974
LPUFA[6]	0.075[ab]	0.121[a]	0.077[a]	0.122[a]	0.071[b]	0.129[b]	-0.769	0.977
[n-3]LPUFA[7]	0.037[a]	0.059[ab]	0.038[a]	0.060[a]	0.036[a]	0.065[ab]	-0.525	0.982
[n-3]LPUFA/FA[8]	$4.41 \cdot 10^{-3a}$		$4.37 \cdot 10^{-3ab}$		$4.06 \cdot 10^{-3 b}$		0.969	
LPUFA/FA[8]	$9.01 \cdot 10^{-3a}$		$8.85 \cdot 10^{-3ab}$		$8.06 \cdot 10^{-3 b}$		0.980	
UFA	1.69[a]	2.72[a]	1.98[b]	3.15[b]	2.09[c]	3.77[c]	0.909	0.997
ΣFA	8.3[a]	13.2[α]	8.7[ab]	13.5[α]	8.9[a]	15.7[β]	0.560	0.461
*c9t11*CLA	0.064[a]	0.102[a]	0.074[b]	0.120[b]	0.099[c]	0.180[c]	0.212	0.208
*t10c12*CLA	0.011[a]	0.018[a]	0.013[c]	0.021[b]	0.013[c]	0.024[b]	0.959	0.958
Isomer ratio[9]	5.80[a]		5.70[a]		7.75[b]		0.926	
*cc*CLA[10]	$1.0 \cdot 10^{-3a}$	$1.5 \cdot 10^{-3a}$	$1.5 \cdot 10^{-3 a}$	$2.4 \cdot 10^{-3 a}$	$1.4 \cdot 10^{-3 a}$	$2.5 \cdot 10^{-3 a}$	0.978	0.820
*tt*CLA[10]	$3.5 \cdot 10^{-3a}$	$5.8 \cdot 10^{-3a}$	$3.9 \cdot 10^{-3 ab}$	$6.2 \cdot 10^{-3 a}$	$4.6 \cdot 10^{-3 b}$	$8.3 \cdot 10^{-3 b}$	0.980	0.981
SFA/UFA[11]	3.94[a]		3.39[b]		3.24[b]		-0.880	
$\Delta 4_{index}$[12]	0.27[ab]		0.26[a]		0.28[b]		0.831	
$\Delta 6_{index}$[12]	0.028[a]		0.024[ba]		0.020[b]		-0.985	
Elongase[13]	0.462[a]		0.442[a]		0.460[a]		0.0856	

[1] means in rows not sharing the same letter are significantly different: [a,b] $P<0.05$; [A,B] $P<0.01$; differences at [α,β] $P<0.1$ are indicated as tendencies; [2] the daily production (g) of assayed fatty acids in goat milk; [3]atherogenic SFA index (A_{index}) (Ulbricht and Southgate, 1991); [4] thrombogenic SFA index (T_{index}) (Ulbricht and Southgate, 1991); [5] the concentration sum of *cis11*C18:1 and *trans11*C18:1; [6] long-chain polyunsaturated fatty acids - the concentration sum: *c11c14*C20:2, *c11c14c17*C20:3, *c5c8c11c14*C20:4, *c5c8c11c14c17*C20:5, *c7c10c13c16c19* C22:5 and *c4c7c10c13c16c19*C22:6 (*c,t* - the abbreviations for *cis* and *trans*); [7] [n-3]LPUFA – the content sum: *cllc14c17*C20:3, *c5c8c11c14c17*C20:5, *c7c10c13c16c19*C22:5 and *c4c7c10c13c16c19*C22:6; [8] [n-3]LPUFA/FA and LPUFA/FA - the concentration ratio of n-3LPUFA to the sum of assayed fatty acids (ΣFA) and the concentration ratio of LPUFA to ΣFA; [9] the concentration ratio (r) of *c9t11*CLA and *t10c12*CLA, i.e. r = *c9t11*CLA/*t10c12*CLA; [10] *cc*CLA, *tt*CLA - *c,c* and *t,t* isomers of CLA, respectively; [11] the concentration ratio of saturated fatty acids (SFA) to UFA, i.e. SFA/UFA; [12] Δ4-desaturase and Δ6-desaturase indexes calculated based on the concentrations of fatty acids in milk: $\Delta 4_{index}$ = *c4c7c10c13; c16c19*C22:6/(*c4c7c10c13c16c19*C22:6 + *c7c10c13c16c19*C22:5); $\Delta 6_{index}$ = *c6c9c12*C18:3/(*c6c9c12*C18:3 + *c9c12*C18:2); [13] the elongase index calculated based on the concentrations of fatty acids in milk; elongase = *c7c10c13c16c19*C22:5/(*c7c10; c13c16c19*C22:5 + *c5c8c11c14c17*C20:5); [14] $r_{mg/ml}$, r_g - the correlation coefficients between crude protein intake and the concentration (mg/ml) of all assayed FA and the daily production (g) of all assayed FAs in milk, respectively

medium level of protein most effectively elevated the concentration of *c11*C18:1 (the substrate upon which Δ9-desaturate acts). Similarly, the increase in protein content in the diet was followed by the statically significant effect ($P<0.05$) to increase the concentration and daily production of the concentration sum of *c11*C18:1 and *t11*C18:1 in milk. As expected, the changes of the concentration and daily production of *t11*C18:1 and *c11*C18:1 in milk are consistent with changes of the concentration and daily production of *c9t11*CLA and *cc* isomer mixture of CLA containing *c9c11*CLA in milk (i.e. the product of Δ9-desaturation of *c11*C18:1) (Czauderna et al., 2003).

The increase of the content of dietary protein (from 11.4 to 16.9%) caused an increase in the concentration and daily production of *t10c12*CLA and *tt*CLA ($P<0.05$). Therefore, we stated that increasing the protein concentration in the diet resulted in improving the nutritional quality of milk due to increases in the concentration of CLA isomers, particularly *c9t11*CLA. The highest protein level (16.9%) in the diet caused highest increase in the concentration ratio of *c9t11*CLA to *t10c12*CLA. Observed increased the concentration ratio of *c9t11*CLA to *t10c12*CLA was probably accounted for by a lowest yield of linoleic acid isomerization to *t10c12*CLA in a rumen of goats fed the diet containing the highest concentration of protein.

The results of the present work confirmed that the protein level in the diet has a minor influence on the concentration and daily production of LPUFA as well as n-3LPUFA in milk. Observed small decrease in the ratios of n-3LPUFA/ΣFA and LPUFA/ΣFA in milk, especially when 16.9% protein was supplemented, might be an effect of ΣFA elevation in the milk and a minor reduction of a capacity of Δ6-desaturase and elongase (Table 2). Therefore, the concentration of LPUFA (the products of Δ4-, Δ6-desaturates and elongase) in milk decreased when the diet with the highest protein level (16.9%) was fed to goats. The increase in the protein level in the diet caused increase ($P<0.05$) in the concentration and daily production of unsaturated fatty acids (UFA) in milk. Moreover, in our study significant decreased ($P<0.05$) the SFA/UFA ratio in milk was stated when the diet was supplemented with 13.3 or 16.9% protein in comparison with the lowest protein treatment. Considering above observations, we suggest that the increase of the amount of protein in the diet was reflected in the decreasing the yield of the biohydrogenation of unsaturated fatty acids in goat rumen.

CONCLUSIONS

Increasing the protein content in the diet improves the health promoting properties of milk, because milk fat contains higher concentrations of unsaturated

fatty acids (UFA), especially *c9t11* and *cc* isomers of conjugated linoleic acid. We hypothesized that decreasing the yield of biohydrogenation in a rumen of goats fed diets containing higher content of protein is mainly responsible for an increase of the concentrations of these fatty acids. In consequence, the concentration of *c11*C18:1 and *t11*C18:1 (incomplete biohydrogenation products of UFA) in milk is positively correlated with the content of protein in the goat diet. We suggest that increasing the dietary protein decreased the capacity of desaturations; this effect resulted in lower concentrations of *pro*-healthy LPUFA in milk. The protein level in the diet has a negligible influence on the concentration *pro*-healthy n-3LPUFA in milk.

REFERENCES

Andrade P.V.D., Schmidely P., 2006. Effect of duodenal infusion of *trans*10,*cis*12CLA on milk performance and milk fatty acid profile in dairy goats fed high or low concentrate diet in combination with rolled canola seed. Reprod. Nutr. Develop. 46, 31-46

Chichlowski M.W., Schroeder J.W., Park C.S., Keller W.L., Schrimek D.E., 2005. Altering fatty acids in milk by including canola seed in dairy cattle diets. J. Dairy Sci. 88, 3084-3094

Chilliard Y., Ferlay A., 2004. Dietary lipid and forage interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Develop. 44, 467-492

Chilliard Y., Ferlay A., Rouel J., Lamberet G., 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86, 1751-1770

Czauderna M., Kowalczyk J., Korniluk K., Wąsowska I., 2007a. Improved saponification followed by mild base- and acid-catalyzed methylations is a useful method for quantifying fatty acids, with special emphasis on conjugated dienes. Acta Chromatogr. 18, 59-71

Czauderna M., Kowalczyk J., Wąsowska I., Niedźwiedzka K.M., 2003. Determination of conjugated linoleic acid isomers by liquid chromatography and photodiode array detection. J. Anim. Feed Sci. 12, 369-382

Czauderna M., Kowalczyk J., Żebrowska T., Michalski J.P., Wąsowska I., 2007b. Response of milk fatty acid profile to various protein levels in goat diets. J. Anim. Feed Sci. 16, Suppl. 2, 548-553

Leiber F., Kreuzer M., Nigg D., 2005. A study on the cause for the elevated n-3 fatty acids in cows' milk of Alpine origin. Lipids 40, 191-202

Nudda A., Battacone G., Usai M.G., Fancellu S., Pulina G., 2006. Supplementation with extruded linseed cake affects concentrations of conjugated linoleic acid and vaccenic acid in goat milk. J. Dairy Sci. 89, 277-282

Rioux V., Catheline D., Bouriel M., Legrand P., 2005. Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5n-3 and C20:3n-6 in the rats. Reprod. Nutr. Develop. 45, 599-612

Ulbricht T.L.V., Southgate D.A.T., 1991. Coronary heart disease: seven dietary factors. Lancet 338, 985-992