Milk and cheese fatty acid profiles in Alpine goat fed green maize forage

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ABSTRACT. Twenty lactating Alpine goats were randomly allocated to two treatment groups to investigate the effect of feeding green maize on the fatty acid profile of milk and cheese. The control goats were fed lucerne hay *ad libitum*; the other group was fed green maize plants *ad libitum*; additionally, all animals received 350 g per day per goat concentrate of the same composition. The green maize diet considerably increased the rumenic acid concentration in milk (0.44% vs 0.60%; $P < 0.01$) and in cheese (0.46% vs 0.78%; $P < 0.01$) and increased the n-3 fatty acids in milk (0.55% vs 1.05%; $P < 0.001$) and in cheese (0.62% vs 1.04%; $P < 0.01$). In addition, the n-6/n-3 ratio was also more favourable in the experimental group (6.04 vs 1.88 in milk, $P < 0.01$; 4.72 vs 2.43 in cheese, $P < 0.001$). It is concluded that the milk and cheese from goats fed green maize are more beneficial in human nutrition than the milk produced by animals fed lucerne hay.

Introduction

The typical modern diet is characterized by a high intake of n-6 polyunsaturated fatty acids (PUFA) and low intake of n-3 PUFA. A high ratio of n-6/n-3 PUFA is a risk factor for coronary heart disease, especially formation of blood clots leading to heart attacks (Enser et al., 1998). The recommended intake is an n-6/n-3 ratio of less than 4 (Simopoulos, 2004). Another beneficial compound of milk is rumenic acid (*c9t11*CLA isomer). It has a range of positive health properties such as anticarcinogenic (Ip et al., 1991) and antiatherogenic effects (Nicolosi al., 1997). Pasture diets have been associated with a high content of conjugated linoleic acid and n-3 fatty acids of milk fat, because grass is rich in α-linolenic acid (Cabiddu et al., 2005). Although a number of studies on the effects of pasture and, in some cases, green forages (Addis et al., 2005) on milk fatty acids, especially on rumenic acid content, have been conducted in ruminant species, literature reports on goat milk and cheese are rather limited (Tsiplakou et al., 2006; Galina et al., 2007; Pajor et al., 2009) compared with those on cows. Unfortunately, during the summer, herbage quantity and quality parameters (e.g., protein content and energy concentration) of pastures decline due to the reproduction phase of grass, consequently, animals need to be provided some additional feed, which in practice means hay-based feeding. In this way, decreases in the milk rumenic acid and n-3 fatty acid contents are observed (e.g., Tsiplakou et al., 2006). One of the possible ways of manipulating the fatty acid contents is by feeding harvested green forages in confinement. We hypothesized that the goat milk fatty acid profile could be improved when green maize was fed instead of a hay-based diet. It is well known that this plant has a high crop yield

and energy content but changes markedly during the vegetation period. Moreover, it is widely cultivated throughout the world and easily fits into green fodder production systems of small-scale farms. Nonetheless, little is still known about the green maize fatty acid contents and the effect of green maize on milk fatty acid composition. The aim of this study was to investigate the effect of feeding green maize on the fatty acid profile of goat milk and cheese.

Material and methods

Experimental animals

The study was carried out in a typical Hungarian goat small enterprise farm on the Great Plain (Kiskunfelegyhaza, Bács-Kiskun County, Hungary). Twenty Alpine goats [average DIM (days in milking) = 110 ± 6.9 days] were randomly allocated to two treatment groups. The groups were balanced for parity and time of kidding. The diets were adjusted to the NRC (2007) recommendations of energy and protein requirements for dairy goats (body weight 60 kg; 2.0 kg of milk per day). The control group (n = 10 goats) was kept indoors and fed *ad libitum* medium quality lucerne hay (crude protein 15.2% of dry matter), the goats from the other group (n = 10) were kept indoors and fed *ad libitum* harvested green maize plants. Additionally, all goats were fed on average 350 g concentrate per goat daily (40% barley, 20% wheat, 20% maize and 20% wheat bran). Both groups had the same composition of the grain mix, which was given twice a day in equal amounts. Daily feed intake (denoted as kg of dry matter) and refusals of forages were measured. A commercial vitamin (A, D_3, E) and trace-mineralized salt block was provided free choice to goats.

The investigation was started in early July and lasted 5 weeks, which encompassed the first 3 weeks as the period of adaptation to the diet and the last 2 weeks as the experimental period. Green maize (vegetation growth stage: beginning of tasseling) was harvested and offered once daily at 8.00 a.m. into self-unloading cages. The samples (n = 3 per forage) of the green maize, lucerne hay and grain mix were collected during the last experimental week. Ingredients, chemical composition, intake, and fatty acid profile of diets are shown in Table 1.

The individual milk samples were collected from animals once a week during the first 2 weeks of the experimental period at 6.00 a.m. and 6.00 p.m., the milk samples were frozen and stored at –20°C until further analysis. Before laboratory investigation, milk samples gathered twice a day were combined for analysis of chemical composition.

Table 1. Ingredients, daily intake, chemical composition and fatty acid profile of the diets

Component	Control diet	Experimental diet
Ingredients, % in DM		
green maize	—	86.0
lucerne hay	85.5	—
concentrate mix	14.5	14.0
daily intake, kg in DM	2.13	2.21
Chemical composition, g · kg⁻¹ DM		
NEl, MJ	5.69	6.20
crude protein	150.05	118.56
MPE [1]	88.11	76.06
MPN [2]	106.47	85.22
crude fat	18.46	25.04
crude fibre	303.22	221.88
crude ash	63.44	65.11
N-free extractives	465.53	571.45
Fatty acids, %		
C12:0	0.36	0.66
C14:0	0.93	0.78
C14:1c9	0.05	0.04
C16:0	30.12	27.79
C16:1c9	1.61	0.86
C18:0	4.43	2.86
C18:1c9	6.36	5.95
C18:2n-6	25.59	28.4
C18:3n-3	19.22	27.03

[1] MPE − metabolizable protein − energy; [2] MPN − metabolizable protein − nitrogen

Cheese processing

A total of 20 kg of goat milk per group was processed into cheese (about 200 g per cheese) at 2, 5, 8, 11 and 14 days throughout the experimental period. The soft cheese was processed by the description shown in Table 2. One soft cheese per batch from each group was taken for chemical analysis. The cheese samples were collected after a 1-week-ripening period. Samples were analysed for fatty acid content as described below.

Table 2. Description of soft cheese manufacturing

Characteristic of processing	
1	Raw milk heating up to 31°C
2	Addition bacterial culture (1.5%) and $CaCl_2$ (15 g · 100 l milk⁻¹), 20 min incubation
3	Addition bacterial commercial rennet, 60 min coagulation time
4	Scooping and draining (wheat size)
5	Post heating up to 35°C, 20 min
6	Formation of cheeses and moulding in block of ~200–300 g
7	Press: 12 h, pressure: self weight, turning: 3x
8	Salt bath: duration: 1 h, cc: 22%, temperature: 15°C
9	Ripen: 17–18°C, 85% relative humidity for 1 week

Chemical analysis

The forage samples (n = 3) were analysed for dry matter, crude protein, crude fat, crude fibre, crude ash and metabolizable protein according to the procedure of the Hungarian Feed Codex (2004).

Fat, protein, lactose and total solids without fat contents of milk were determined using a Milko-Scan device (Combi Foss 5000 apparatus, Foss Electric, Denmark).

The milk and cheese fat were dissolved in a sodium hydroxide–methanol solution and re-esterified to methyl esters according to the AOAC (1990) method using boron trifluoride (BF_3). Methyl esters of fatty acids were determined by gas chromatography using a Shimadzu GC 2010 apparatus (Japan) with a flame ionization detector (FID) and column (CP-SIL-88, 100 m × 0.25 mm × 0.2 μm). The split injection ratio was 50:1. The column oven temperature was held at 80°C for 0 min, then programmed at a rate of $2.5°C \cdot min^{-1}$ up to 205°C and held for 20 min and then increased again to 225°C at $10°C \cdot min^{-1}$, and held for 5 min. The injector and detector temperatures were 270°C and 300°C, respectively. Helium was used as the carrier gas, applying a flow rate $28 \; cm \cdot s^{-1}$. Peaks were identified on the basis of the retention times of standard methyl esters of individual fatty acids (Mixture Me 100, Larodan Fine Chemicals AB, Sweden). The proportions of the individual acids were calculated by the ratio of their peak area to the total area of all observed acids.

Statistical analysis

Statistical analysis was processed by the SPSS 14.0 software package (Shapiro-Wilk test for normality distribution, F test for equality of variances, t-test, and Welch's corrected t-test).

Results

The daily forage intake (dry matter, kg) was similar between the two diets (control: 2.13 kg, experimental: 2.21 kg), but the daily milk yield during the investigation was lower ($p < 0.05$) in control goats at 2.1 kg, compared with 2.4 kg milk yield for experimental goats. Milk composition was significantly affected by diet. The lucerne hay diet caused lower ($p < 0.05$) fat ($3.02 \; g \cdot 100 \; g^{-1}$), protein ($2.89 \; g \cdot 100 \; g^{-1}$), lactose ($4.60 \; g \cdot 100 \; g^{-1}$) and non-fat solids ($7.55 \; g \cdot 100 \; g^{-1}$) contents compared with milk from green maize-fed goats (3.22, 2.94, 4.69 and $7.87 \; g \cdot 100 \; g^{-1}$, respectively) (data not presented in Table). Thus, the diets significantly influenced milk composition during the investigated period.

The results of fatty acid analysis of milk and cheese samples are presented in Table 3.

The green maize diet significantly increased the concentrations of short-chain fatty acids (butyric, caproic and caprylic), stearic (C18:0), α-linolenic (C18:3), eicosapentaenoic (C20:5), docosahexaenoic (C22:6), total polyunsaturated and n-3 fatty acids, as well as significantly decreased those of lauric (C12:0), miristoleic (C14:1), palmitoleic (C16:1), linoleic (C18:2), and n-6 fatty acids in milk and cheese.

In contrast, the concentrations of capric acid (C10:0), palmitic acid (C16:0), oleic acid (C18:1), total saturated fatty acids and total monounsaturated fatty acids in milk and cheese samples did not show any significant differences during the experimental period.

In the present study, the n-6/n-3 ratios equalled 6.04 and 1.88 in milk and 4.72 and 2.43 in cheese samples from animals receiving hay and green maize treatments, respectively.

Feeding green maize positively affected the concentration of rumenic acid in milk and cheese samples. In milk, the rumenic acid concentrations were 0.44% and 0.60%, in cheese, 0.46% and 0.78% for the control and experimental diets, respectively.

Discussion

Feeding green maize significantly influenced the yield and composition of goat milk. A higher energy concentration, lower metabolizable protein content, as well as a slight N imbalance (MPN-MPE) were detected in the experimental diet. Positive N balance is important in early lactation, but in mid-lactation on a medium milk production level, it is not crucial. The milk production, fat and protein contents were higher in goats from the experimental group. This could be a result of the higher energy concentration, as energy is provided to support protein synthesis. Nevertheless, it is well known that goat milk fat and protein contents are affected by many factors, such as diet, breed, parity and stage of lactation (Kuchtik et al., 2008; Novotna et al., 2009). Soryal et al. (2004) in Alpine goat, and Pajor et al. (2009) in Hungarian Native goat, found that feeding green fodder (pasture) slightly increased the milk fat, protein and non-fat solids compositions. Milk composition is important for breeders because it determines the composition of cheese.

The experimental feeding regime increased the concentrations of short-chain fatty acids in samples of milk and cheese. Increases in butyric, caproic and caprylic fatty acids have been observed.

Table 3. Effect of green maize supplementation on fatty acid profile and conjugated linoleic acid isomers of goat milk and cheese (mean ± SD), %

Fatty acids	Milk samples				Cheese samples			
	control diet	experimental diet	SEM	*P*	control diet	experimental diet	SEM	*P*
C4:0	0.59	1.58	0.13	***	0.68	1.05	0.07	*
C6:0	1.27	2.24	0.14	***	1.38	2.32	0.16	***
C8:0	2.11	2.83	0.13	**	2.15	2.63	0.09	*
C10:0	9.80	10.14	0.31	N.S.	8.70	9.03	0.11	NS
C12:0	5.47	4.03	0.31	*	4.89	3.96	0.17	**
C14:0	12.25	10.28	0.35	**	12.09	11.50	0.15	N.S.
C14:1c9	0.19	0.13	0.16	*	0.19	0.15	0.01	*
C16:0	32.80	30.77	0.71	N.S.	30.77	29.30	0.42	NS
C16:1c9	0.86	0.37	0.06	***	0.65	0.51	0.03	**
C18:0	8.23	11.63	0.60	**	8.28	11.39	0.61	*
C18:1c9	16.96	18.64	0.53	N.S.	21.91	22.27	0.17	NS
C18:2n-6	2.98	1.79	0.17	***	2.62	2.28	0.07	*
C18:3n-3	0.41	0.80	0.05	***	0.45	0.79	0.07	**
C20:4n-6	0.22	0.12	0.01	***	0.28	0.19	0.02	*
C20:5n-3	0.03	0.07	0.01	**	0.02	0.06	0.01	**
C22:5n-3	0.09	0.14	0.01	***	0.13	0.14	0.00	NS
C22:6n-3	0.02	0.04	0.00	***	0.02	0.05	0.01	**
SCFA	13.76	16.79	0.63	*	12.91	15.02	0.38	**
SFA	74.71	75.92	0.61	N.S.	72.38	73.12	0.59	NS
MUFA	20.91	19.13	0.58	N.S.	23.54	22.49	0.31	NS
PUFA	4.38	4.94	0.13	*	4.08	4.40	0.07	*
n-6	3.23	1.94	0.18	***	2.91	2.47	0.09	*
n-3	0.55	1.05	0.06	***	0.62	1.04	0.08	**
n-6/n-3	6.04	1.88	0.55	***	4.72	2.43	0.42	***
*c9t11*CLA	0.44	0.60	0.03	*	0.46	0.78	0.06	**
*t10c12*CLA	0.09	0.10	0.01	N.S.	0.09	0.11	0.01	NS
CLA, total	0.53	0.70	0.03	**	0.55	0.89	0.06	**
*c9t11*CLA, %	82.63	85.35	0.53	**	83.65	88.16	1.03	*

NS – non significantly difference; * – $p < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ – significant differences between two groups; SCFA – short chain fatty acids (C_4-C_{10}); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; CLA – conjugated linoleic acid

The results obtained in the present investigation differ from those diets which rich in PUFA such as linseed supplemented concentrate (Zhang et al., 2006). Higher values of polyunsaturated fatty acids from the diet inhibit *de novo* fatty acid synthesis in the mammary gland (Couvrer et al., 2006). This is confirmed by the decreased medium-chain fatty acid content (C12:0 and C14:0 fatty acids) due to the higher secretion of long-chain fatty acids from the blood (dilution effect; Chilliard et al., 2000). In contrast, short-chain fatty acids are not influenced by a high PUFA content, as these fatty acids are synthesized in the mammary gland from acetic acid, which originates from the rumen. These results suggest that the green forage positively affected rumen function and as well as acetic acid production.

The experimental diet significantly increased the concentrations of α-linolenic and other n-3 fatty acids in milk and cheese. In our study, the green maize α-linolenic acid concentration in total fat was 30.7%, the n-6/n-3 ratio was less than 1. Grass is also rich in α-linolenic acid – n-3 fatty acids account for about 50% of the total fat in grass (Cabiddu et al., 2005). During drying, however, decreases in forage PUFA and n-3 fatty acid concentrations occur due to oxidation and leaf losses (Doreau and Poncet, 2000; Dewhurst et al., 2006). Moreover, because of rumen biohydrogenation, a high proportion of polyunsaturated fatty acids, such as linoleic and linolenic acid, are biohydrogenated in the rumen. Both fatty acids are hydrogenated to stearic acid: the relative percentage of C18:0 was significantly increased in green maize-fed goats (11.63% in milk and 11.39% in cheese) compared with the control group (8.23% in milk, 8.28% in cheese). Nevertheless, linoleic acid (C18:2) is biohydrogenated to a smaller extent than linolenic (C18:3) acid (Lee et al., 2003). On the other hand, reduction of linoleic acid in the rumen was faster with green forage than with dried forage (Buccioni et al., 2008). On

the basis of these factors, feeding green maize can increase the concentrations of n-3 fatty acids such as α-linolenic acid and decrease the concentrations of n-6 fatty acids such as linoleic acid in milk and cheese. The milk and cheese from the experimental goats had a higher α-linolenic acid content (0.41% vs 0.80% in milk; $P < 0.001$; 0.45% vs 0.79% in cheese; $P < 0.01$). In the present study, n-6/n-3 ratios of 6.04 and 1.88 and 4.72 and 2.43 were found for milk and cheese samples of goats that fed control and experimental diets, respectively. The low n-6/n-3 ratio in the milk and cheese of goats that received green maize is in keeping with the new recommendations for human nutrition. α-linolenic acid is the precursor of n-3 PUFAs such as eicosapentaenoic acid (EPA) ($C_{20:5}$ n-3) and docosahexaenoic acid (DHA) ($C_{22:6}$ n-3), which are required for many metabolic processes in humans and effectively prevent coronary heart disease (CHD). The concentrations of α-linolenic, eicosapentaenoic and docosahexaenoic acids were higher in the milk and cheese from green maize-supplemented animals than in those from the goats fed hay.

The rumenic acid concentrations in milk and cheese were affected by dietary treatments. This *c9t11*CLA isomer was higher in milk (0.44% vs 0.60 %; $p < 0.05$) and cheese (0.46% vs 0.78%; $P < 0.01$) from goats in the experimental group than in the control group. Other authors have reported that the diet – such as one with a high protein level in the dry matter of forage (Czauderna et al., 2010) and supplemental sunflower or soyabean fats (Titi et al., 2011) – can increase the rumenic acid concentration in milk. In addition, the contents of water and fermentable carbohydrates in the forage have significant effects on microbiological fermentation and pH in the rumen. Rumen pH has an important role in maintaining a viable environment suitable for *Butyrivibrio fibrisolvens* (Tsiplakou et al., 2008). Microbial fermentation is influenced by fermentable carbohydrates; it is well known that green maize has a high content of this component, so it creates a favourable rumen environment for rumenic acid-producing bacteria, as reported by Kelly et al. (1998).

In the milk and cheese samples, about 82.6%– 88.2% of the total conjugated linoleic acid isomers were the *c9t11*CLA isomer. This is in agreement with the literature reports. Chin et al. (1992) found rumenic acid percentages ranging from 75% to 90% in dairy products. Feeding green maize considerably increased the rumenic acid percentage in milk (82.6% vs 85.4%; $P < 0.01$) and cheese (83.7% vs 88.2%; $p < 0.05$), respectively. The *t10c12*CLA content of milk and cheese samples did not show any significant differences during experimental period.

The *t10c12*CLA isomer is produced in the rumen by minor bacterial populations or an alternative biohydrogenation pathway (Tsiplakou et al., 2006).

Conclusions

There were significant differences in milk composition and fatty acid contents between the two treatments. Feeding green maize resulted in significantly higher concentrations of rumenic acid (*c9t11*CLA isomer) and n-3 fatty acids such as α-linolenic, eicosapentaenoic (EPA) ($C_{20:5}$ n-3) and docosahexaenoic (DHA) ($C_{22:6}$ n-3) acids, which are of special interest in terms of promoting human health. Based on our results, it can be concluded that a disadvantageous fatty acid profile due to absence of pasture herbage availability can be improved by the use of a green maize-based diet. Nevertheless, further research is needed to investigate the developing of goat milk EPA and DHA fatty acid contents from other PUFA sources, such as micro-alga; data on feeding micro-alga supplements are limited, however, although it is well known that the micro-alga species are rich source of PUFAs.

Acknowledgements

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