Impact of crude protein content in silage and concentrate on protein and fatty acid profiles in bovine milk

M. Johansson[1], O. Placha[1], J. Pickova[1], A. Andrén[1], G. Zamaratskaia[1], E. Spörndly[2], M. Åkerstedt[1]

[1]Department of Food Science, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden
[2]Department of Animal Nutrition and Management, Kungsängen Research Centre, Uppsala, Sweden

ABSTRACT: Two concentrates, one protein-rich and one based on cereals, were combined with two silages with a crude protein content of 17 and 13% of dry matter (DM), respectively to give four different diets for dairy cows. Milk content of caseins (α_{S1}-, α_{S2}-, β-, and κ-casein) and whey proteins (α-lactalbumin (α-LA) and β-lactoglobulin (β-LG)) and the fatty acid profile of milk were analyzed before the start and on four occasions during the experiment. Milk analyses showed that diet had no influence on the protein profile of the milk. However, a significant increase of α-linolenic acid, 13 and 39%, was obtained on the high protein concentrate feed and on the silage higher in crude protein, respectively. Cows on the protein-rich concentrate diet increased the proportion of conjugated linoleic acid by 53%. Linoleic acid was not affected by the diet.

Keywords: milk proteins; feed protein; clover; casein; CLA; α-linolenic acid; capillary electrophoresis

The feeding regime for dairy cows differs between conventional and organic production. In organic production a higher percentage of roughage is required. In the rules for organic production within the European Union, roughage should represent at least 60% of the cow's diet with the exception of the first three months where 50% roughage is accepted (European Commission, 2008). Dairy producers also need to supply the cows with organically produced feed to fulfil the rules for organic production (European Commission, 2008). However, the amount of organically produced feed is limited on the market, resulting in increased interest in feed self-sufficiency on organic dairy farms. In addition, if organic dairy producers want to feed protein supplements, the supplements must also be organically produced, which increases the price of these products. Furthermore, supply of organic protein supplements on the market is limited, as is the choice of suitable protein feeds that can be organically produced on the dairy farm. It is therefore of great interest to investigate alternative feeding regimes without the use of protein concentrate supplements to evaluate effects on production parameters such as milk composition.

High quality roughage combined with a concentrate consisting of only cereals could be an interesting feeding regime for organic dairy producers. This alternative feeding strategy is economically attractive since cereals can often be grown on the dairy farm. It is therefore important to evaluate the possibility of replacing protein concentrates to some extent with high-quality silage containing a high proportion of legumes such as clover (Rosati and Aumaitre, 2004). Earlier studies have shown that clover has a positive influence not only on feed intake, but also on milk yield (Bertilsson et al., 2002; Bertilsson and Murphy, 2003). It is well known that the fatty acid (FA) profile of milk can

be influenced by changes in the feed (Palmquist et al., 1993; Chilliard and Ferlay, 2004), while the total protein content is affected to a much lesser degree (Theurer et al., 1995; Olmos Colmenero and Broderick, 2006). A drawback with this feeding regime is a lack of protein in the diet. Cows fed only high quality roughage *ad libitum* without any concentrates had a considerably lower milk yield compared with a standard feeding regime with concentrates (Johansson and Holtenius, 2008).

In addition to milk yield, it is important that the milk quality remains at a high level with regard to content of economically important components such as protein and fat. In general, dietary composition is known to modify milk protein yield rather than protein concentration (Theurer et al., 1995; Olmos Colmenero and Broderick, 2006). Most previous studies have focused either on the effects of diet on total N content rather than true protein (casein) content or on content of caseins and whey proteins, while studies on individual proteins are scarce. However, such studies are important as the proportion of κ-casein (κ-CN) is recognized to increase cheese yield (Wedholm et al., 2006; Hallén et al., 2010). In addition, the dominant whey protein, β-lactoglobulin (β-LG), improves gel strength in acid-induced milk gels, i.e. the consistency and mouth feel of fermented products such as yoghurt (Hallén et al., 2009). Therefore it is crucial to evaluate the influence of diet on the concentration of individual milk proteins.

In addition, there is a growing interest in the fatty acid composition of milk, especially conjugated linoleic acid, C18:2*c*9*t*11 (CLA), and polyunsaturated fatty acids due to their effect on human health (Chilliard and Ferlay, 2004). The concentration of linoleic acid, C18:2n6 (LA), and that of alpha-linolenic acid, C18:3n3 (ALA), is dependent on the diet, as these FA cannot be synthesized by the bovine metabolism (MacGibbon and Taylor, 2006). However, most of these fatty acids provided in the diet undergo bacterial modification in the rumen, depending on the feed matrix (Leiber et al., 2005), with LA being the main precursor of CLA. It has been suggested that if the levels of these FA could be increased, milk could be considered a functional food (Bauman and Griinari, 1999; Mattila-Sandholm and Saarela, 2003).

The aim of this study was to determine how individual caseins and whey proteins and the unsaturated fatty acids LA, ALA, and CLA in bovine milk were affected by diets with two types of concentrates (cereal only vs. cereals + protein-rich feeds) combined with two silages with a crude protein content of 17 and 13% DM, respectively.

MATERIAL AND METHODS

Animals, management, and feeding

This study of the effect of diet on milk components was conducted as part of a larger production experiment with 36 cows of the Swedish Red breed on the experimental farm belonging to the Swedish University of Agricultural Sciences (SLU) in Uppsala. It is known that udder health can affect milk composition (Kelly et al., 2006). Therefore, it was important to include only healthy animals in the analysis and only cows with an average somatic cell count below 100 000 cells/ml calculated over all sampling occasions were used in this milk composition analysis. A total of 17 cows (average somatic cell count of 28 000 cells/ml) fulfilled the requirements for low somatic cell count and were thus chosen to study the effects of the four experimental diets (see below) on milk protein composition and on the content of unsaturated fatty acids LA, ALA, and CLA in milk. The cows were all in the early part of lactation when the first sampling was conducted, with an average of 62 days in milk (DIM). The majority of the cows were multiparous (10 cows) and the remaining 7 cows were primiparous. The experiment lasted for 20 weeks including a pre-period and experimental period. The transition from one type of the food into another was continuous and completed over a time period of one week.

The animals were milked in a barn with automatic milking (AM) with the DeLaval Voluntary milking system (VMS) (DeLaval, Tumba, Sweden). Cow traffic was monitored with smart gates where cows moving from the area with resting cubicles to the eating area were directed to milking if 6 h had passed since the latest milking. Cows with less than 6 h since previous milking could go directly to the feeding area where concentrates were available in concentrate feeders and roughages were fed in individual feed troughs that monitored the intake of each cow. The cows were identified by transponders in both concentrate and roughage feeders ensuring that each cow got the right type and amounts of concentrate and roughage according to treatment group and pre-determined ration.

After a pre-period when the cows were fed silage dominated by early harvested grass and a pre-experimental concentrate containing 23% oats, 23% barley, 20% peas, 12% rapeseed cake, 9% beet fibre, 7% wheat bran, 3% crushed whole rapeseed, and 3% minerals, the cows were randomized into four experimental groups. The treatments consisted of two silages and two concentrates in a 2 × 2 factorial design. The two silage treatments consisted of grass silages with a high (silageH) or low (silageL) content of crude protein, 17 and 13%, respectively. The higher crude protein content in silageH was obtained by mixing 62% (on DM basis) of a grass-dominated silage with 38% pure of red clover (*Trifolium pratense*) silage before feeding. SilageL was a grass-dominated silage with a clover content of only 5% of DM. The grass fraction in the silages consisted of two-thirds meadow fescue (*Festuca pratensis*) and one-third timothy (*Phleum pratense*) on DM basis and they were harvested from the same field. The cows in the treatment concH were given both cereal pellets and protein-rich pellets as en extra supplement while the cows in the treatment concL were given cereal pellets only (concL). The cereal pellets contained 36% barley, 34% wheat, 25% oats, 2% molasses, and 3% minerals while the protein-rich pellets contained 47% soybean expeller, 16% rapeseed cake, 15% oats, 12% crushed whole rapeseed, 4% roasted soybean, 3% wheat, 1% molasses, and 2% minerals. Thus, each group was fed one of the four diet combinations: silageH + concH, silageH + concL, silageL + concH, and silageL + concL with 5, 3, 4, and 5 cows in the four diet combinations (i.e. treatments), respectively. The number of animals on the four diets studied was not completely balanced due to the pre-requisite to only include cows with a low somatic cell count in the analysis. Silage was fed *ad libitum* and the cereal and protein supplement was given to the cows in accordance with their milk yield.

Sampling

The milk was sampled at three consecutive milkings twice in the pre-period and four times after the diet transition (samplings 1–4). The milk samples from the three individual milkings during each sampling were pooled for each cow. As these cows were milked in a milking robot with an average milking frequency of 2.4 (range 1.8–2.8) milkings per day, three samples correspond to the milk produced during at least a 24-hour period. These milk samples were stored at –80°C prior to analysis of individual milk proteins and fatty acids. The amount of energy corrected milk (ECM) was calculated according to the equation by Sjaunja et al. (1990).

Feed samples of the silages were taken daily during the period when milk samples were collected and pooled over a 14-day period. Chemical analysis was performed on two pooled silage samples for the pre-experimental period, and on four pooled samples during the experimental period. Feed samples of the cereal pellets and the protein pellets were sampled two times per week and pooled over a 4-week period to check that the level of ash and crude protein corresponded to the values of chemical supplied by the feed factory. Conventional chemical analysis of feed composition including the feed content of dry matter, ash, crude protein, and NDF as well as calculations of the feed content of metabolizable energy were performed as described by Bertilsson and Murphy (2003). The chemical composition and nutrient content of the feeds are presented in Table 1.

Protein analyses by capillary electrophoresis

In total, four proteins from the casein family (α_{S1}-casein (α_{S1}-CN), α_{S2}-casein (α_{S2}-CN), β-casein (β-CN), and κ-casein (κ-CN)) and two whey proteins (α-LA and β-LG) were analyzed. Protein analyses were carried out using Capillary Electrophoresis (CE) Liquid Chromatograph Agilent G 1600AX (Agilent Technologies Co., Kista, Sweden), controlled by Chemstation software (Version A 10.02). Separation of the proteins was performed as described by Åkerstedt et al. (2012), using unfused silica standard capillary column, 50 μm inner diameter, 40 cm active length (Chrom Tech, Märsta, Sweden). The column was pre-conditioned with Milli-Q water (Milli-Q water system) (Millipore, Bedford, USA) for 10 min, followed by a 5-min pause. This was followed by flushing the column with running buffer for 20 min. Finally, the column was rinsed with Milli-Q water for 10 min and run buffer for 15 min. Separations were carried out at 45°C and a linear voltage gradient from 0 to 25 kV for 3 min, followed by constant voltage at 25 kV. Before each separation, the column was flushed

Table 1. Chemical composition and nutrient content of the feeds used during the pre-period before experimental start and during the experimental period. Two silages with a crude protein content of 17% (SilageH) or 13% (SilageL) were fed. Silages were combined with two types of concentrate supplements based on cereals (CerealS) or based on protein rich components (ProtS). See paper for details of feeding regimes in the four treatment groups. Values reported for concentrates and concentrate supplements were obtaind from feed manufacturer. Values for silage in pre- and experimental period are based on analysis of 2 and 4 feed samples, respectively and are reported with standard deviation in parenthesis

	Pre-period		Experimental period			
	concentrate	silage	ProtS	CerealS	SilageH	SilageL
Dry matter (DM) (%)	88.0	34.3 (0.81)	91.3	87.0	34.5 (1.19)	35.7 (1.52)
Content (g kg/DM)						
Ash	62	83 (1.60)	84	63	87 (4.0)	76 (7.90)
Starch	350	–	99	559	–	–
Crude fat	60	–	130	34	–	–
NDF	253	–	183	205	414 (19.40)	471 (13.90)
Crude protein	172	138 (0.30)	338	118	171 (2.90)	133 (0.30)
AAT	82	74	160	84	73	74
PBV	26	11	99	–21	46	7
Energy (MJ kg/DM)	13.2	11.7 (0.04)	15.5	13.0	11.4 (0.19)	11.6 (0.11)

NDF = neutral detergent fibre, AAT = amino acids absorbed in the small intestine calculated according to the Swedish feed tables (Spörndly, 2003), PBV = protein balance value calculated according to the Swedish feed tables (Spörndly, 2003)

with Milli-Q water for 3 min, followed by run buffer for 5 min. Sample solutions were injected at the anode by pressure injection at 50 mbar for 7 s.

Preparation of sample solutions and capillary electrophoresis buffers

The sample buffer (pH 8.6) consisted of 0.167M-(tris)hydroxymethylaminomethane (TRIS), 0.067M-ethylene-diamine-tetraacetic acid disodium salt dihydrate (EDTA), 0.042M-MOPS, 6M-urea-trisodium-dehydrate solution, 0.017M-D,L-dithiothreitol (DTT), and w/w 0.05% methylhydroxyethylcellulose 3000 (MHEC) (all from Sigma-Aldrich Inc., St. Louis, USA), dissolved in the urea solution. The sample solutions were filtered through a 0.45 µm nylon membrane filtre before analyses by CE. The running buffer (pH 3.0) consisted of 0.19M-monohydrate citric acid, 0.02M-trisodium citrate dehydrate, 6M-urea, and w/w 0.05% MHEC 3000 (all from Sigma-Aldrich), dissolved in the urea-trisodium-dehydrate solution. For both buffers, urea solution dissolved in water was prepared with 2 g/100 ml of ion exchange resin (AG® 501-X8 and Bio-Rex® MSZ 501(D) Mixed Bed Resin) (Bio-Rad Laboratories Inc., Hercules, USA) and stirred until the conductivity reached below 2 µS. Both buffers were filtered through a 0.45 µm filter paper (Durapore® membrane filters) (Millipore, Solna, Sweden). Buffers and samples were stored at –20°C prior to analysis.

Before preparation, the milk samples were defatted by centrifugation at 3222 *g* (Eppendorf 5810R) (VWR International, Stockholm, Sweden) at 4°C for 12 min and incubated in a water bath at 42°C for 30 min. The sample solution was prepared by mixing 300 µl milk sample with 700 µl sample buffer. After mixing, the sample solution was left at room temperature for 1 h.

Identification of capillary electrophoresis peaks

Identification of peaks was based on milk protein standards, α_S-CN, β-CN, κ-CN, α-LA, and β-LG (all from Sigma-Aldrich Inc., St. Louis, USA), and confirmed with previously published electropherograms (Miralles et al., 2003). Multiple peaks around the main peak of α_{S2}-CN were assigned to α_{S2}-CN according to Heck et al. (2008).

Lipid extraction of milk

Milk lipids were extracted following the method of Hara and Radin (1978). In brief, pooled samples (1 ml) were mixed with 5 ml HIP (hexane : isopropanol 3 : 2 v/v). Non-lipids were removed by adding 2.5 ml 6.67% (w/v) sodium sulphate (both Sigma-Aldrich Inc., St. Louis, USA) to each sample. Samples were vortexed and centrifuged at 3222 *g* at 18°C for 5 min. The upper lipid phase was separated and evaporated under nitrogen gas. Remaining lipids were re-suspended in 0.5 ml hexane and stored at –80°C until analysis. A microbalance scale (Mettler Toledo, Greifensee, Switzerland) was used to measure the lipid content.

Preparation of fatty acid methyl esters (FAME)

Total milk lipids were methylated according to the procedure of Appelquist (1968). To each sample, 17 : 1 fatty acid (Larodan Fine Chemicals, Malmö, Sweden) was added as internal standard for quantification. Then 2 ml 0.01M NaOH in dry methanol (both Sigma-Aldrich Inc., St. Louis, USA) were added to each sample of 2 mg total lipid, shaken and placed in a 60°C heating block for 10 min. Next, 3 ml of BF_3 reagent (20% boron trifluoride-methanol complex) (Merck, Darmstadt, Germany) were added and the samples were reheated for 10 min. Once cooled to room temperature, 2 ml 20% NaCl (Sigma-Aldrich Inc.) and 2 ml hexane were added. Test tubes were shaken and allowed to stand for 20 min to separate the layers. The lipid was evaporated under nitrogen gas, solved in hexane, and stored at –80°C until analysis.

Gas chromatography analysis

Fatty acid profile of the milk from all treatments was analyzed and the polyunsaturated fatty acids LA, ALA, and CLA were selected for investigation. FAME were analyzed with a gas chromatograph CP3800 (Varian AB, Stockholm, Sweden) equipped with flame ionisation detector (FID) and split injector and fitted with a fused silica capillary column BPX 70 (SGE, Austin, USA), column length 50 m, ID 0.22 mm, film thickness 0.25 µm. The samples (1 µl) were injected by a CP8400 autosampler (Varian AB, Stockholm, Sweden), split mode. A split ratio of 1 : 10 was used. Column temperature was programmed to start at 158°C for 5 min and then increase by 2°C/min from 158 to 220°C and remain at 220°C for 8 min. Injector and detector temperatures were 230 and 250°C, respectively. The fatty acid profile was expressed as a percentage of identified fatty acids. Peak areas were integrated using STAR chromatography workstation software (Version 5.5, 2007). The carrier gas was helium (22 cm/s, flow rate 0.8 ml/min) and the make-up gas was nitrogen.

Statistical analyses

The statistical analyses were performed using Statistical Analysis System (Version 9.3, 2008). The MIXED Procedure of SAS (Proc Mixed) was used. A mean value over the four sampling occasions for each response variable was the base for the statistical analysis. The model used included the effects of the treatments, i.e. the factors concentrate and silage including their interaction. The covariate used was the value obtained for the response variable in the pre-period before the

Table 2. Effect of concentrate type and silage type on milk yield, milk fat, and milk protein content. Least Squares Means with standard error in parenthesis and significance levels for effect of concentrate and effect of silage

	ConcH[1]	ConcL[2]	SilageH[3]	SilageL[4]	Effect of concentrate	Effect of silage
Milk (kg)	39.0 (1.88)	30.5 (2.01)	34.1 (2.02)	35.4 (1.88)	**	ns
ECM (kg)	37.5 (1.75)	31.0 (1.88)	33.4 (1.87)	35.1 (1.74)	*	ns
Fat (%)	3.77 (0.154)	4.47 (0.166)	4.10 (0.157)	4.15 (0.147)	*	ns
Protein (%)	3.03 (0.058)	3.23 (0.062)	3.18 (0.063)	3.08 (0.059)	*	ns

[1]concentrate high based on protein supplements and cereals, [2]concentrate low based on only cereals, [3]silage high containing 17% crude protein and 38% clover, [4]silage low containing 13% crude protein and 5% clover

ECM = energy corrected milk (Sjaunja et al., 1990)

*$P < 0.05$, **$P < 0.01$, ns = non-significant

Table 3. Effect of concentrate type and silage type on milk protein components expressed as mg/ml milk. Least Squares Means with standard error in parenthesis and significance levels for effect of concentrate and effect of silage

	ConcH[1]	ConcL[2]	SilageH[3]	SilageL[4]	Effect of concentrate	Effect of silage
α_{S1}-casein	9.8 (0.373)	10.9 (0.399)	10.1 (0.398)	10.6 (0.371)	*	ns
α_{S2}-casein	2.1 (0.121)	2.4 (0.129)	2.2 (0.128)	2.2 (0.120)	ns	ns
κ-casein	3.4 (0.072)	3.6 (0.078)	3.6 (0.079)	3.5 (0.073)	*	ns
β-casein	10.7 (0.617)	12.3 (0.663)	10.9 (0.690)	12.2 (0.639)	ns	ns
β-lactoglobulin	2.2 (0.052)	2.1 (0.055)	2.2 (0.056)	2.2 (0.053)	ns	ns
α-lactalbumin	1.5 (0.088)	1.6 (0.094)	1.6 (0.097)	1.5 (0.090)	ns	ns

[1]concentrate high based on protein supplements and cereals, [2]concentrate low based on only cereals, [3]silage high containing 17% crude protein and 38% clover, [4]silage low containing 13% crude protein and 5% clover
*P < 0.10, ns = non-significant

experiment started. The interaction between the factors concentrate and silage was non-significant for all response variables and was therefore excluded from the final model. Thus, the final model used was the following simple Proc Mixed model:

Y = concentrate + silage + covariate

RESULTS

Milk yield and milk content of fat and protein

Milk yield and ECM were significantly higher (Table 2) when cows were fed the protein-rich concH compared with cereal-based concL. In contrast, fat and protein contents were significantly higher for concL. As a consequence, the difference between concentrate treatments in ECM production was somewhat lower than the difference in kg milk. Silage had no significant effects either on milk yield, ECM, fat or protein.

Protein composition of milk

The impact of concentrate type and silage type on individual proteins in the milk are presented in Table 3. There were no significant effects for the caseins and whey proteins when comparing cows that were fed the two different concentrates (concH and concL) and two different silages (silageH and silageL).

Fatty acid composition of milk

Effects of concentrate type and silage type on the content of LA, ALA, and CLA in milk fat (% of total fatty acids) are presented in Table 4. There were no significant effects either of concentrates or silages in LA content in the milk. For ALA the high protein concentrate feed (concH) and the silage higher in crude protein (silageH) resulted in by 13 and 39% higher contents of this FA, respectively (Table 4). Cows on the concH diet increased the proportion of CLA by 53% (Table 4). No significant effect of silages on CLA was observed.

Table 4. Effect of concentrate type and silage type on the content of C18:2n-6, C18:3n-3, and C18:2*c*9*t*11 in milk fat (% of total fatty acids). Least Squares Means with standard error in parenthesis and significance levels for effect of concentrate and effect of silage

	ConcH[1]	ConcL[2]	SilageH[3]	SilageL[4]	Effect of concentrate	Effect of silage
C18:2n-6	1.73 (0.117)	1.39 (0.127)	1.63 (0.127)	1.49 (0.117)	*	ns
C18:3n-3	0.85 (0.023)	0.75 (0.025)	0.93 (0.024)	0.67 (0.022)	**	***
C18:2*c*9*t*11	0.75 (0.040)	0.49 (0.043)	0.64 (0.043)	0.61 (0.040)	***	ns

[1]concentrate high based on protein supplements and cereals, [2]concentrate low based on only cereals, [3]silage high containing 17% crude protein and 38% clover, [4]silage low containing 13% crude protein and 5% clover
*P < 0.10, **P < 0.01, ***P < 0.001, ns = non-significant

DISCUSSION

The aim of this study was to determine how individual caseins and whey proteins and the unsaturated fatty acids LA, ALA, and CLA in milk were affected by high-protein and low-protein diets for dairy cows.

The cows were identified by transponders in both concentrate and roughage feeders ensuring that each cow got the right type and amounts of concentrate and roughage according to treatment group and pre-determined ration. Thus, the effects of feed on milk composition could be studied over a longer time period without imposing restrictions on cow movement or natural behaviour, especially with regard to feeding patterns.

Although the total protein content in the milk was significantly higher for the animals fed the concL diet, no effects of feed on the individual milk protein components were observed in this study. However, we observed a tendency for increase of α_{S1}-casein and κ-casein when cows were fed concL. This is in agreement with other studies showing that the diet has limited effect on protein synthesis connected with protein concentration in milk (Theurer et al., 1995; Olmos Colmenero and Broderick, 2006).

When viewing the concentration of unsaturated fatty acids LA, ALA, and CLA of the total milk fatty acids, concH caused a significant increase in the proportion of unsaturated fatty acids ALA and CLA. Tendency for a significant increase in concentration of LA of total milk FA was also perceived. As shown in Table 1, the cows fed concH were given a feed that was not only higher in protein content, but also in fat content. Thus, the effect on milk FA could be due to the higher fat content in the concH diet. This would be in agreement with Chilliard et al. (2000), who showed that milk fat composition could be modulated by lipid supplementation. However, concL resulted in a significantly higher total milk fat content (Table 2) due to an effect of this diet on the FA that are produced in the udder because the cows fed concL had a higher concentration of FA with up to 16 carbons in the chain.

In the present study milk yield was significantly higher when cows were fed the concH compared with the concL diet (39.0 and 30.5 kg milk respectively) (Table 2). In contrast to the results of Bertilsson and Murphy (2003), the silageH with its high clover content did not increase milk yield in the present study, even though the crude protein content was approximately by 4% higher in silageH compared with silageL (Tables 1 and 2). The lack of response to the silage with the higher protein content is somewhat surprising as a certain beneficial effect on milk yield of supplying silage with a higher protein content could be expected, especially for the cows on the cereal based concentrate (concL). However, it seems that the higher crude protein content in the silage could not compensate for the lack of high quality protein that is found in concentrate components such as soybean and rapeseed products (Tables 1 and 2).

It seems that the effect on the levels of LA, ALA, and CLA was not solely due to a diet with higher fat levels when cows were fed the concH. We showed that the cows fed concH had significantly lower fat content in the milk (Table 2). The combined effect of a lower fat content in milk (Table 2) but a higher proportion of the milk fat being unsaturated (Table 4) for cows on treatment concH evens out the differences between treatments concH and concL with regard to the amounts of ALA and CLA in the milk.

Our starting hypothesis was that silageH, which contained 38% red clover, would increase the levels of LA, ALA, and CLA. In spite of this, only the level of ALA was significantly higher (Table 4). It was in agreement with Dewhurst et al. (2003) and Al-Mabruk et al. (2004) who found that red clover silage has positive effect on ALA, as was also the case in the present study. In contrast to Lee et al. (2006) the silage types in the present study had no significant effects on LA and CLA.

Leiber et al. (2005) showed that secondary clover constituents can inhibit microbial biohydrogenation, allowing some ALA to escape from the rumen intact and appear in the milk fat. This may be one of the reasons why the milk of cows fed a clover-rich silage (SilageH) had a higher proportion of ALA than that of cows fed a silage containing only 5% clover. This result confirms the potential for modifying the concentration of this FA in milk by feeding silage with a higher content of red clover. However, in the case of LA and CLA, no significant effect of silageH was observed. This was also documented by Kalač and Samková (2010) who showed that the silage seems to affect the content of CLA less than other forages.

The hypothesis concerned the possible effects of concH. The results showed that CLA concentration was significantly higher when concH was fed (Table 2).

The major constituent of this concentrate was soybean and rapeseed. As CLA is formed in the rumen as an intermediate product in the digestion of dietary LA (Chilliard et al., 2000), the concH could cause increasing CLA concentration.

CONCLUSION

The results of this study demonstrate that it is difficult to obtain responses to dietary changes in milk protein content based on protein-rich feed supplements. It also appears that effects on the protein composition in milk cannot be obtained by feeding silages with different proportions of crude protein with associated differences in red clover content. For fatty acids, only the content of ALA in milk FA was significantly higher by feeding silage with high clover content. Increased ALA and CLA concentrations in the milk FA were associated with feeding regimes involving a protein-rich concentrate supplement and not with high-quality silage containing a high proportion of legumes such as clover.

REFERENCES

Åkerstedt M., Wredle E., Lam V., Johansson M. (2012): Protein degradation in bovine milk caused by *Streptococcus agalactiae*. Journal of Dairy Research, 79, 297–303.

Al-Mabruk R.M., Beck N.F.G., Dewhurst R.J. (2004): Effects of silage species and supplemental vitamin E on the oxidative stability of milk. Journal of Dairy Science, 87, 406–412.

Bauman D.E., Griinari J.M. (1999): Biosynthesis of CLA and its incorporation into meat and milk of ruminants. Journal of Animal Science, 77, 117.

Bertilsson J., Murphy M. (2003): Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass and Forage Science, 58, 309–322.

Bertilsson J., Dewhurst R.J,.Tuori M. (2002): Effects of legume silages on feed intake, milk production and nitrogen efficiency. Sonderheft 234, Special issue, Landbauforschung Völkenrode, FAL Agricultural Research, 39–45.

Chilliard Y., Ferlay A. (2004): Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reproduction Nutrition Development, 44, 467–492.

Chilliard Y., Ferlay A., Mansbridge R.M., Doreau M. (2000): Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Annales de Zootechnie, 49, 181–205.

Dewhurst R.J., Evans R.T., Scollan N.D., Moorby J.M., Merry R.J., Wilkins R.J. (2003): Comparison of grass and legume silages for milk production. 2. *In vivo* and *in sacco* evaluations of rumen function. Journal of Dairy Science, 86, 2612–2621.

European Commission (2008): Commission Regulation (EC) No 889/2008 of 5 September 2008. Official Journal of the European Union, L250, 1–84.

Hallén E., Allmere T., Lundén A., Andrén A. (2009): Effect of genetic polymorphism of milk proteins on rheology of acid-induced milk gels. International Dairy Journal, 19, 399–404.

Hallén E., Lundén A., Allmere T., Andrén A. (2010): Casein retention in curd and loss of casein into whey at chymosin-induced coagulation of milk. Journal of Dairy Research, 77, 71–76.

Hara A., Radin N.S. (1978): Lipid extraction of tissues with a low toxicity solvent. Analytical Biochemistry, 90, 420–426.

Heck J.M.L., Olieman C., Schennink A., van Valenberg H.J.F., Visker M.H.P.W., Meuldijk R.C.R., van Hooijdonk A.C.M. (2008): Estimation of variation in concentration, phosphorylation and genetic polymorphism of milk proteins using capillary zone electrophoresis. International Dairy Journal, 18, 548–555.

Johansson B., Holtenius K. (2008): Organic milk production based entirely on grassland feeds. In: Hopkins A., Gustafsson T., Bertilsson J., Dalin G., Nilsdotter-Linde N., Spörndly E. (eds): Biodiversity and Animal Feed – Future Challenges for Grassland Production. Proc. 22nd General Meeting of the European Grassland Federation, Uppsala, Sweeden, 825–827.

Kalač P., Samková E. (2010): The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. Czech Journal of Animal Science, 55, 521–537.

Kelly A.L., O'Flaherty F., Fox P.F. (2006): Indigenous proteolytic enzymes in milk: a brief overview of the present state of knowledge. International Dairy Journal, 16, 563–572.

Lee M.R.F., Connelly P.L., Tweed J.K.S., Dewhurst R.J., Merry R.J., Scollan N.D. (2006): Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 2. Lipids. Journal of Animal Science, 84, 3061–3070.

Leiber F., Kreuzer M., Nigg D., Wettstein H.R., Scheeder M.R.L. (2005): A study on the causes for the elevated n-3 fatty acids in cow's milk of alpine origin. Lipids, 40, 191–202.

MacGibbon A.K.H., Taylor M.W. (2006): Composition and structure of bovine milk lipids. In: Fox P.F., McSweeney P.L.H. (eds): Advanced Dairy Chemistry. Volume 2: Lipids. 3rd Ed. Springer, New York, 1–41.

Mattila-Sandholm T., Saarela M. (2003): Functional Dairy Products. 1st Ed. Woodhead Publishing Ltd., Cambridge, UK.

Miralles B., Ramos M., Amigo L. (2003): Influence of proteolysis of milk on the whey protein to total protein ratio as determined by capillary electrophoresis. Journal of Dairy Science, 86, 2813–2817.

Olmes Colmenero J.J., Broderick G.A. (2006): Effect of dietary crude protein concentration on ruminal nitrogen metabolism in lactating dairy cows. Journal of Dairy Science, 89, 1694–1703.

Palmquist D.L., Beaulieu A.D., Barbano D.M. (1993): Feed and animal factors influencing milk fat composition. Journal of Dairy Science, 76, 1753–1771.

Rosati A., Aumaitre A. (2004): Organic dairy farming in Europe. Livestock Production Science, 90, 41–51.

Sjaunja L.O., Baevre L., Junkkarinen L., Pedersen J., Setälä J. (1990): A Nordic proposal for an energy corrected milk (ECM) formula. In: Proc. 27th Biennial Session of the International Committee for Animal Recording (ICAR), Paris, France, 156–192.

Spörndly R. (ed.) (2003): Feed tables for ruminants 2003. Report No. 257, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden, 1–96. (in Swedish)

Theurer C.B., Huber J.T., Santos F.A.P. (1995): Feeding and managing for maximal milk protein. In: Proc. Southwest Nutrition and Management Conference, University of Arizona, Tucson, USA, 59–67.

Wedholm A., Larsen L.B., Lindmark-Månsson H., Karlsson A.H., Andrén A. (2006): Effect of protein composition on the cheese-making properties of milk from individual dairy cows. Journal of Dairy Science, 89, 3296–3305.

Received: 2012–10–19
Accepted after corrections: 2013–03–21

Corresponding Author

Monika Johansson, Ph.D., Swedish University of Agricultural Sciences, BioCenter, Department of Food Science, SE-750 07 Uppsala, Sweden
Tel. +46 18 672 041, fax +46 18 672 995, e-mail: monika.johansson@slu.se