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Original Article

Effect of feeding whole soybean and linseed on milk and Parmigiano Reggiano cheese lipid fraction

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Summary. Aim of this study was to assess the effects of feeding whole soybean flakes and whole extruded linseed to dairy cows on milk lipid fraction and Parmigiano Reggiano cheese produced from it; specifically, PUFA and CLA contents were evaluated. For 14 weeks, a herd of 145 cows received a diet with a daily supplementation of 1 kg of whole soybean flakes and 0.3 kg of whole extruded linseed. In the pre-trial period, cows received an isoproteic ration devoid of both seeds. From 20 cows selected from the herd, individual milk, bulk morning milk and vat milk samples of the entire herd were taken every 14 days. After 24 months of aging, cheese samples were taken from twelve cheese wheels, obtained from milk produced at 0, 4, 8, 10, 12 and 14 weeks on the same days as bulk milk and vat milk samples were made. Fatty acid composition of the lipid fraction was determined in all milk and cheese samples. Feeding whole extruded linseed and soybean flakes has determined a decrease in short-chain fatty acids content and an increase in total CLA concentration of the milk. Bulk milk, vat milk and aged cheese analysis samples confirmed a decrease of short-chain fatty acids content in milk and revealed a decrease in n-6/n-3 fatty acid and in saturated/unsaturated fatty acid ratios. In conclusion, feeding a ration including whole soybean flakes and extruded linseed can improve the nutritional characteristics of milk and of Parmigiano Reggiano cheese, particularly with respect to their lipid fractions.

Key words: Dairy cows, milk quality, cheese quality, polyunsaturated fatty acids (PUFA), conjugated linoleic acids (CLA)

Introduction

According to the World Health Organization, cardiovascular diseases today represent the greatest threat to human health and will continue to do so in the upcoming years. Standing alongside them in terms of severity and dissemination are various forms of cancer, whose incidence is progressively increasing (1). It is by now widely acknowledged that the combined actions aimed at changing people's lifestyles and diet are effective as part of the strategy to prevent the aforesaid pathologies, in particular those affecting the cardiovascular system. In this context, a foremost role in nutrition is played by lipids, understood both as a source of energy and polyunsaturated fatty acids (PUFA) and a balanced source of PUFA of the n-6 and n-3 series (2).

The fatty acid (FA) content and balance of dietary lipids are qualifying elements of the nutritional properties and health properties of food. As regards milk and dairy products in particular, an adequate presence of long-chain polyunsaturated fatty acids (LCPUFA) and the balance between the n-6 and n-3 series represent characterizing elements of great importance (3).
The factors capable of modulating milk fat quality have been addressed by numerous publications (4-9) and are associated with breed, lactation phase and

diet. Milk lipid fraction of ruminants, it is well known that saturated fatty acids (SFA), with different chain lengths, prevail over PUFA in the balance among the FA of triglycerides.

Among the LCPUFA, conjugated linoleic acids (CLA) are a series of positional isomers of linoleic acid (C18:2), which have biological activities that are beneficial for health. The properties of CLA include (10-14) anti-carcinogenic, anti-atherogenic, anti-adipogenic, immunomodulating and anti-diabetic effects. However, the biological activity of CLA that has been most thoroughly investigated is that tied to the protection against the onset of some types of tumors, such as breast and colon cancer, as well as stomach and skin cancer.

It has been demonstrated that the inclusion of naturally CLA-enriched butter in rat diets is capable to reduce the incidence of experimentally induced tumors by about 50% during the development of the mammary gland, thus providing the same results as obtained with administration of synthetic CLA (15). Naturally CLA-enriched butter also contains a higher level of vaccenic acid (C18:1 trans11), which represents another intermediary of ruminal biohydrogenation; since vaccenic acid can be metabolized to CLA through the action of the enzyme Δ9-desaturase, it represents an additional source of CLA for the body (16).

CLA are synthesized into rumen as intermediate products of the process of hydrogenation of LCPUFA, and in particular of linoleic acid (17); as noted, CLA are also produced in the udder and in tissues by desaturation of vaccenic acid due to Δ9-desaturase (18).

In consideration of the continuing ban on using animal meals (fish meal in this specific case) in the diet of ruminants that are a source of food for humans, the dietary enrichment of milk in LCPUFA, and CLA in particular, can be pursued by:

- feeding fresh forages and/or oilseed crops, that are raw materials rich in linoleic and α-linolenic acid and precursors of both LCPUFA and CLA;
- administering microencapsulated feed supplements, with rumen-protected inert material, consisting of substrates rich in PUFA (linseed oil, fish oil, algae, etc.) in order to prevent them from undergoing the alterations otherwise induced by the ruminal bacterial microflora and thus make them available for intestinal absorption (19, 20).

The awareness that, in human nutrition, diets with a generally low n-6/n-3 ratio are to be preferred, has placed zootechnical products obtained from grazing ruminants (4, 8, 21) in a favorable light, as these animals receive diets rich in fresh forages containing good amounts of CLA precursors (3, 21, 22). However, a challenge arises in intensive (or conventional) farming, where animals are fed cereal-rich diets and only rarely significant amounts of fresh forages. Under these conditions, dietary intake of n-3 precursors is significantly reduced, whilst the n-6 FA content increases (23). Given the objective difficulty of reducing dietary intakes of n-6 FA (in any case, sources of corn with a different linoleic acid concentration (24) and of sunflower with a high oleic content (25) are already available on the market), it is evident that the desired decrease in n-6/n-3 ratio can be achieved by increasing n-3; among other things, this would make the characteristics of the milk obtained in intensive farming more similar to those of milk from grass-fed cows.

Aim of this study was to evaluate the possibility of increasing of n-3 PUFA and CLA contents in milk, and thus in the Parmigiano-Reggiano cheese produced from it, by supplementing cows diet with raw materials (whole soybean flakes and whole extruded linseed) containing α-linolenic acid and other CLA precursors, within the limits imposed by Parmigiano Reggiano PDO (Protected Designation of Origin) cheese production regulations (26).

Materials and Methods

The University of Bologna Scientific Ethics Committee on Animal Experimentation Examined and approved the experimental protocol (no. 13825-X/10 All.: 67).

Animals and diet

This study was conducted at the Caretti family dairy farm (S. Giovanni in Persiceto, Bologna), situated within the Parmigiano Reggiano Production Area; milk is produced in accordance with the Parmigiano Reggiano cheese production regulations (26), and is processed in their cheese-making facility (Caseificio S. Angelo – Registration no. 3552).

During the pre-trial period (4 weeks), 145 cows of the Italian Friesian breed had been milked there, in a loose housing barn and divided equally among four boxes; 20 of the lactating cows were selected for milk sampling (5 cows per box) based on age and order of lactation (Table 1).

Prior to the start-up of the trial, representative samples of the batches of forages that would be used during the study were taken with the aid of a core sampler.

The entire herd was fed using the total mixed ration (TMR) technique, with unifeed provided *ad libitum*. Diet was based using high quality forages (mainly alfalfa hays) supplemented with concentrate produced on the farm, based on: corn, barley, sorghum, bran, sugar-beet pulp, soybean meal, minerals and vitamins.

TMR and concentrate compositions, optimized using CPM software (CPM-Dairy V3 Program), are shown respectively in Tables 2 and 3. During the trial period (14 weeks), cows were fed a TMR with the same protein composition as normally used, supplemented with 1 kg/head/d of whole soybean flakes and 0.3 kg/head/d of whole extruded linseed (Table 2).

Table 1. Selected animals features at the beginning of the trial (average ± s.d.)

Animals	n	20
Delivery	n	3.14 ± 2.01
Days of lactation	n	71.48 ± 39.66
Body Condition Score	pts	2.80 ± 0.14
Milk yield	kg/head/d	39.74 ± 6.81
Fat	%	3.39 ± 1.07
Protein	%	3.31 ± 0.29
Casein	%	2.60 ± 0.24
Lactose	%	5.05 ± 0.13
Urea	mg/dl	24.63 ± 3.65

Measured parameters

The average daily feed intake (DMI) for each of the four boxes was recorded weekly, as a difference between TMR delivered and refusal before the next TMR was supplied; samples of the TMR were collected, immediately after unloading, for subsequent analytical determinations to be performed in the Department of Veterinary Medical Sciences laboratories.

Every month, individual milk production of the 20 selected cows was measured and evening milk samples were collected on a biweekly basis, for the determination of fat, protein, casein, lactose and urea content, as well as the FA composition.

Every two weeks, a bulk milk sample, from each box, was taken in the morning upon delivery of milk to cheese-making facility and a vat milk sample, from four vats: these samples were analyzed to determine fat, protein, casein, lactose and urea content and FA composition.

During the trial, to evaluate cheese yield, the amount of milk processed in four different cheese vats was recorded weekly. Weights of the "twin cheese wheels" obtained after 24 hours of processing were subsequently recorded; moreover, samples of vat milk and of cream naturally skimmed were collected on a weekly basis. Data concerning bulk milk, vat milk and cream samples were recorded throughout the trial period.

After 2 years of aging, on a monthly basis, two of the twelve twin cheese wheels obtained from four

Table 2. Composition of total mixed ration technique

Period		Pre-trial	Experimental
Alfalfa hay	kg/head/d	10.00	10.00
Alfalfa hay (1° cut)	kg/head/d	5.00	5.00
Whole soybean flakes	kg/head/d	-	1.00
Whole extruded linseed	kg/head/d	-	0.30
Concentrate	kg/head/d	12.30	11.00
Water	kg/head/d	6.00	6.00

Table 3. Composition of concentrates

Period		Pre-trial	Experimental
Corn	%	40.00	40.00
Sorghum	%	20.00	20.00
Barley	%	6.90	14.90
Sugar beet pulps	%	11.00	11.00
Soft wheat bran	%	11.00	11.00
Soybean meal	%	8.00	-
Sodium bicarbonate	%	1.00	1.00
Sodium chloride	%	0.90	0.90
Calcium carbonate	%	0.70	0.70
Magnesium oxide	%	0.30	0.30
Trace elements and vitamins	%	0.20	0.20

experimental vats were sampled for determining fatty acid profile of lipid fraction of the aged cheese.

Chemical analyses

TMR samples were analyzed to determine dry matter, nitrogen, ether extract and starch content in accordance with AOAC guidelines (27; method 930.15 for dry matter, method 954.01 for nitrogen, method 920.39 for ether extract, and method 920.40 for starch). Fiber fraction was determined using the method described by Van Soest et al. (28), whereas the method of Licitra et al. (29) was used to determine soluble nitrogen.

With regard to analytic determinations performed on milk samples, fat, protein, casein, lactose and urea contents were quantified using a Milko Scan (Foss Electric, Hillerod, Denmark). FA composition was determined by gas chromatography (GC), using the method described by Christie (30), on extracted lipids [method described by Folch et al. (31) and methylated lipids (method ISO 15884)].

GC was performed with a Fisons HRGC MEGA2 series 8560 gas chromatograph with autosampler and Fisons Chrom-Card software; the chromatograph is equipped with a 100-meter-long Varian, CP-SIL 88 WCOT Fused Silica capillary column with an internal diameter of 0.25 mm and internal film thickness of 0.2 μm. The initial column temperature was 45°C for 8 min, followed by an increase of 12°C/min, isotherm at 173°C for 47 min, increase of 4°C/min and final isotherm at 220°C for 30 min; injector temperature 250°C and (FID) detector temperature 270°C. The pressure of the carrier gas (helium) was 215 kPa and 1 microliter of sample was injected with a split ratio of 50:1. The individual FA were expressed as a percentage of total FA.

Statistical analysis

All data obtained were subjected to one-way ANOVA for repeated-measures, with time as the main effect; Dunnett test was used for post hoc analysis. For all individual data, the individual cow was used as experimental unit, the box for feed consumption (n=4); as regards cheese-making data, bulk milk samples (n=4), milk vat samples (n=4), cream and cheese samples (n=4) represented the experimental unit. Differences where $P \leq 0.05$ were considered statistically significant relative to the pre-trial period. Statistica 10.0 software (StatSoft Italia, Vigonza (PD), Italy) was used for the analysis.

Results

Zootechnical and production parameters

Characteristics of the rations: chemical composition of the TMR sampled weekly is shown in Table 4, whereas Table 5 shows the FA content of the pre-trial diet and the experimental one.

Feed intake and individual production: DMI was not influenced by inclusion of whole soybean flakes and whole extruded linseed (24.1 ± 0.9 vs 24.2 ± 1.1 kg d.m./d. in pre-trial and trial periods, respectively).

Milk production of selected cows (Table 6) became significantly lower during trial period as the stage of lactation advanced.

Individual and bulk milk samples quality: the fat content of both individual and bulk milk samples was significantly lower in the period of supplementation, as were urea concentrations, which were lower than in the pre-trial phase (Table 6 and 7); this could be due to the lower solubility of provided proteins.

Table 4. Analytical composition of diets (average ± s.d.)

Period		Pre-trial	Experimental
Analysis	n.	4	14
Dry matter	%	83.45 ± 2.95	80.27 ± 3.16
Protein	%	14.62 ± 0.68	14.40 ± 0.82
Ether extract	%	2.81 ± 0.13	3.71 ± 0.23
Soluble protein	%	3.65 ± 0.31	3.48 ± 0.44
NDIP	%	3.66 ± 0.82	3.41 ± 0.71
ADIP	%	0.83 ± 0.28	0.98 ± 0.22
Ash	%	6.98 ± 0.51	7.84 ± 0.43
NDF	%	38.01 ± 3.03	39.67 ± 2.99
ADF	%	23.51 ± 1.48	22.46 ± 1.78
ADL	%	4.07 ± 0.45	4.29 ± 0.39
Starch	%	23.45 ± 1.89	24.39 ± 2.02
Ca	%	0.73 ± 0.07	0.75 ± 0.08
P	%	0.41 ± 0.05	0.38 ± 0.09

Table 5. Fatty acids composition of diets (% of total fatty acids)

Period	Pre-trial	Experimental
Analysis, n	2	2
C12:0	0.5	0.3
C14:0	1.1	0.7
C16:0	18.1	14.1
C16:1	0.9	0.5
C18:0	2.4	3.2
C18:1trans11	0.1	0.1
C18:1cis	13.6	17.0
C18:2	44.5	39.8
C18:3	15.5	22.2
Others	3.4	2.2

Table 6. Milk yield and quality of the selected animals

Period		Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n	-	40	160	-	-
Milk yield	kg/d	39.49	35.44	1.321	≤0.001
Fat	%	3.58	3.48	0.022	≤0.05
Protein	%	3.26	3.27	0.012	n.s
Casein	%	2.55	2.57	0.009	n.s.
Lactose	%	5.04	5.03	0.005	n.s.
Urea	mg/dl	25.79	24.12	0.315	≤0.01

Table 7. Analytical composition of bulk milk

Period		Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n		8	32	-	-
Fat	%	3.67	3.60	0.021	≤0.05
Protein	%	3.27	3.22	0.029	n.s.
Casein	%	2.48	2.47	0.029	n.s.
Lactose	%	4.96	4.95	0.018	n.s.
Urea	mg/dl	24.23	23.00	0.713	≤0.01

Cheese-making parameters

Vat milk quality and cheese yields: milk fat content in cheese vat decreased in the period of whole soybean and linseed supplementation (Table 8), whereas no significant differences were observed for protein and casein concentrations. Results in terms of cheese yield are similar to those obtained in other trials we conducted on the same farm (32, 33); it is worth pointing out a significant decrease of cheese yield in experimental period, which may be attributed to the lower protein and fat contents of vat milk.

GC determination of fatty acids in milk and cheese

Individual and bulk milk samples: a comparison between the FA concentrations of individual samples in trial period versus pre-trial period (Table 9) shows that the content of short-chain SFA was significantly reduced (P≤0.01). In contrast, α-linolenic acid

Table 8. Analytical composition of vat milk and cheese yield

Period		Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n		8	32	-	-
Fat	%	2.68	2.60	0.021	≤0.05
Protein	%	3.28	3.26	0.029	n.s.
Casein	%	2.54	2.51	0.029	n.s.
Lactose	%	5.03	5.00	0.018	n.s.
Urea	mg/dl	25.33	23.45	0.713	≤0.001
Cheese yield	kg/100kg	8.15	7.80	0.087	≤0.001

Table 9. Fatty acids composition (% of total fatty acids) of selected cows

Period	Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n	40	160	-	-
C12:0	3.98	4.04	0.404	n.s.
C14:0	12.06	12.90	0.953	n.s.
C16:0	33.07	33.89	1.800	n.s.
C18:0	9.76	9.25	1.225	n.s.
C18:1 trans 11	0.47	0.72	0.194	n.s.
C18:1 n-9	19.78	19.14	2.155	n.s.
C18:2 n-6	2.64	2.53	0.207	n.s.
C18:3 n-3	0.67	0.80	0.060	≤0.05
CLA tot	0.33	0.49	0.054	≤0.05
Short chain	7.01	5.65	0.411	≤0.01
Medium chain	22.24	23.21	1.854	n.s.
Long chain	70.75	71.14	1.878	n.s.
Saturated	75.52	75.86	2.612	n.s.
Unsaturated	27.54	27.62	2.271	n.s.
Monounsaturated	23.50	23.39	2.148	n.s.
Polyunsaturated	1.40	1.71	0.091	≤0.01
n-6/n-3	0.08	0.06	0.011	≤0.01

and cis-9, trans-11 CLAs concentrations significantly increased (P≤0.05). Moreover, n-6/n-3 ratio underwent a significant decrease (P≤0.01) compared to pre-trial period. *Cream samples*: dietary treatment had no effect on FA composition of cream (results omitted), that were in line with what reported by Verardo et al. (34).

Milk vat samples: vat milk analysis showed some differences compared to pre-trial period (Table 11); it is worth highlighting, in particular, the reduction (P≤0.05) in linoleic acid content and increase in CLA concentration (P≤0.05), confirming what was observed in individual and bulk milk samples.

Cheese samples after 24 months of aging: cheese lipid fraction analysis (Table 12) revealed a significant decrease in short- and medium-chain SFA (P≤0.05 and P≤0.001, respectively), as well as an increase in unsaturated fatty acids (UFA) (P≤0.01), monounsaturated in particular (P≤0.001).

A significant decrease linoleic and α-linolenic acid concentrations was also observed (P≤0.001); the content of these PUFA in aged cheeses was lower than that in cheese obtained in pre-trial period. With regard to total CLA, significant differences in favor of the trial period were maintained (P<0.05).

The trend in both SFA/UFA and n-6/n-3 FA ratios in aged cheese was particularly interesting: in fact, a statistically significant decrease (P≤0.001) in both ratios was observed.

Discussion

Zootechnical and production parameters

Feed intake and individual production: soybean and linseed supplementation did not negatively impact feed intake, contrary to what was observed by other authors (35), who observed a lower DMI in dairy cows receiving three different amounts (78, 142 and 209 g/kg d.m. of the diet) of whole crushed linseed. DMI recorded during trial is to be considered normal for production levels and type of unifeed fed. Forages were finely chopped in order to limit the possibility for cows

Table 10. Fatty acids composition (% of total fatty acids) of bulk milk

Period	Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n	8	32	-	-
C12:0	4.17	4.63	0.710	n.s.
C14:0	13.64	14.98	1.726	n.s.
C16:0	35.29	31.58	2.617	≤0.05
C18:0	9.18	9.99	1.542	n.s.
C18:1 trans 11	0.48	0.75	0.083	n.s.
C18:1 n-9	22.45	22.58	3.582	n.s.
C18:2 n-6	2.65	2.32	0.199	n.s.
C18:3 n-3	0.73	0.73	0.068	n.s.
CLA tot	0.37	0.50	0.056	≤0.05
Short chain	5.69	6.49	0.532	n.s.
Medium chain	20.92	22.77	2.733	n.s.
Long chain	73.39	70.74	2.969	n.s.
Saturated	71.08	70.91	3.593	n.s.
Unsaturated	28.92	29.09	3.523	n.s.
Monounsaturated	25.02	25.37	0.310	n.s.
Polyunsaturated	3.90	3.73	0.532	n.s.
n-6/n-3	3.01	2.62	0.152	n.s.

Table 11. Fatty acids composition (% of total fatty acids) of the vat milk

Period	Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n	8	32	-	-
C12:0	3.77	4.46	0.458	n.s.
C14:0	12.99	14.99	1.303	n.s.
C16:0	35.26	33.68	2.084	n.s.
C18:0	9.86	9.74	1.276	n.s.
C18:1 trans 11	0.51	0.73	0.177	n.s.
C18:1 n-9	22.16	21.23	2.750	n.s.
C18:2 n-6	2.79	2.26	0.237	≤0.05
C18:3 n-3	0.79	0.73	0.061	n.s.
CLA tot	0.34	0.46	0.050	≤0.05
Short chain	5.99	6.44	0.485	n.s.
Medium chain	19.90	22.20	1.947	n.s.
Long chain	74.11	71.37	2.091	n.s.
Saturated	71.01	72.19	2.572	n.s.
Unsaturated	28.99	27.81	2.572	n.s.
Monounsaturated	24.88	24.14	2.487	n.s.
Polyunsaturated	4.10	3.67	0.299	n.s.
n-6/n-3	2.84	2.62	0.173	n.s.

to select among unifeed and also to increase DMI and could have effect in reducing rates of biohydrogenation associated with UFA, thereby increasing the transit time of feed.

Quality of individual, bulk and vat milk samples: the decrease in lipid content in individual (P≤0.01), bulk (P≤0.05) and vat (P≤0.05) milk samples is in line with what has been reported by other authors (20, 36), who observed a decrease in milk fat in cows fed 2.0 kg of extruded soybean and DHA-based rumen-protected product, respectively. In general, many studies (37; 10, 38, 39) have found a reduction in lipid content of cow's milk when feed supplements containing significant amounts of PUFA are used, and this is attributable precisely to the synthesis of CLA, the trans-10, cis-12 isomer in particular.

The decrease of urea levels in milk we observed could be attributed to the use of lots of hays with particularly high levels of degradable fiber and hence of energy for cellulosolytic bacteria growth, which, as is well known, use ammonia as their principal source of nitrogen: the farm uses hot-air forage dryers (55-60°C) in order to obtain dehydrated forages of excellent quality; data obtained are wholly comparable to the ones we obtained in ours previous trials (40).

GC determination of fatty acids in milk and cheese

Individual, bulk and vat milk samples: Tables 9, 10 and 11 show the FA composition of individual, bulk and VAT milk samples respectively. The decrease of short-chain FA content is in agreement with what was observed by Cavalieri et al. (41), who had fed dairy cows with whole linseed (11.5% of d.m. of unifeed). With regard to the increased concentration of cis-9, trans-11 CLA, similar results were obtained by Moallem (42) consequently the administration of higher amounts (700 g/head/d) of extruded linseed (700 g/head/d) to dairy cows.

Cheese samples after 24 months of aging: the lipid fraction analysis of cheeses (Table 12) revealed a signi-

Table 12. Fatty acids composition (% of total fatty acids) of the Parmigiano Reggiano cheese

Period	Pre-trial	Experimental	Pooled SEM	ANOVA P
Samples, n	2	12	-	-
C12:0	4.37	3.95	0.028	≤0.001
C14:0	12.39	12.08	0.042	≤0.01
C16:0	27.90	29.93	0.048	≤0.001
C18:0	9.08	7.51	0.028	≤0.001
C18:1 trans 11	0.93	1.14	0.033	n.s.
C18:1 n-9	16.23	16.34	0.067	≤0.01
C18:2 n-6	2.76	2.06	0.012	≤0.001
C18:3 n-3	0.65	0.67	0.003	n.s.
CLA tot	0.26	0.38	0.021	≤0.05
Short chain	3.73	3.58	0.032	≤0.05
Medium chain	20.95	20.11	0.081	≤0.001
Long chain	75.32	76.32	0.111	≤0.01
Saturated	61.07	60.18	0.102	≤0.01
Unsaturated	38.93	39.82	0.102	≤0.01
Monounsaturated	35.44	36.95	0.107	≤0.001
Polyunsaturated	3.48	2.87	0.047	≤0.01
n-6/n-3	3.39	3.38	0.029	≤0.001

ficant decrease, compared to pre-trial period, in short- (P≤0.05) and medium-chain (P≤0.001) fatty acids; on the whole, the medium-chain fatty acids content was comparable with that reported by Prandini et al. (43), who evaluated the acidic profile of numerous samples of Grana Padano cheese lipid fractions, produced in different seasons of the year. PUFA content (linoleic and α-linolenic acid) in aged cheeses was lower (P≤0.001) than observed in cheeses produced in pre-trial period. As a possible justification for this unexpected finding, it must be remembered that, in Parmigiano Reggiano production, the long aging (more than 24 months) induces an extremely reducing environment within the cheese, which may be cause saturation phenomena involving UFA. This would confirm how much production technology can influence FA composition of the cheese (44).

A significant increase was observed in total CLA content, likely with what was affirmed by other authors (45) who assessed the effects resulting from the administration of 300 g/head/d of extruded linseed meal; moreover, these FA concentrations are completely in line with what has been reported in the literature for various types of aged PDO cheeses (8).

The trend in both the SFA/ UFA and n-6/n-3 FA ratios in aged cheese (Table 12) was particularly interesting: there was a statistically significant decrease (P≤0.001) in both ratios, and this trend was maintained throughout the trial period. Similar findings were reported by other authors who used whole linseed (46).

Conclusions

The results of this study show that adding whole soybean flakes and whole extruded linseed to the diet of dairy cows may significantly modify the lipid fraction of milk.

In particular, supplementing the diet with soybean and linseed determined a decrease in the milk fat (individual and bulk) and short-chain FA content, an increase n-3 FA relative to n-6 FA content and a significant increase in the total CLA concentration.

The increase in total CLA concentration and their precursors in milk seems to confirm that feeding a diet containing whole soybean flakes and whole extruded linseed leads to an increase in CLA concentration as a result of high dietary amounts of linoleic and α-linolenic acid.

Taking into consideration the entire production chain of Parmigiano Reggiano PDO cheese, from the bulk milk, through natural skimming, to the vat milk and, finally to the aged cheese, it may be affirmed that aging substantially influenced cheese FA composition, which differed from milk it was obtained from. The experimental diet caused about an overall decrease of short- and medium-chain SFA contents, an increase in total CLA amount and a significant improvement in n-6/n-3 and SFA/UFA ratios, thus bringing milk and cheese closer to meet nutritional requirements of wholesomeness increasingly demanded by consumers. In conclusion, these results enable us to affirm that it is possible to modulate milk lipid fraction intended for cheese production by means of a targeted and appropriate diet, whilst remaining within the limits on the use of raw materials imposed by Parmigiano Reggiano PDO cheese production regulations (26).

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