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Fatty acid profile and coagulating ability of milk from Jersey and Friesian cows fed whole flaxseed

Mariangela Caroprese*, Roberta Mancino, Maria Giovanna Ciliberti, Aldo Di Luccia, Barbara La Gatta and Marzia Albenzio

Department of the Sciences of Agriculture, Food and Environment (SAFE), University of Foggia, Via Napoli, 25, 71122 Foggia, Italy

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The experiment was carried out to evaluate the effects of a moderate level of flaxseed administration on milk coagulation properties and fatty acid profile of milk from two different breeds. The experiment was performed on 20 Italian Friesian cows and 20 Jersey cows divided into 2 groups of 10 animals each. The experimental diets were (1) a traditional diet (CON) administrated as unifeed and no supplemental fat and (2) a diet supplemented with 0·5 kg/d of whole flaxseed (FS). Cows were milked twice daily and milk yield was recorded. Milk samples were analysed at 1, 15, and 30 d of the experiment for composition, pH, and milk coagulation properties. To verify the effects of flaxseed administration on the coagulation properties of milk from Friesian and Jersey cows, an electrophoresis study on casein fractions was performed. Milk fatty acid profile can be improved by administrating a moderate level of flaxseed in the diet, however, milk fatty acid profile from Friesian and Jersey cows showed different contents of C18 : 1 *trans*-11, SFA and MUFA. The results demonstrated that milk coagulating ability can be increased by flaxseed administration in both breeds as a result of different aggregation of casein micelles.

Keywords: Milk fatty acid, coagulating ability, Jersey cow, whole flaxseed.

Health-conscious consumers are demanding nutritious food with beneficial features from a human health perspective. The major issue in relation to milk consumption is the milk fatty acid (FA) profile, mainly represented by saturated fatty acids, which account for 62–70% of the total milk fatty acids. However, milk and dairy products are characterised also by the presence of rumenic acid (RA) and its precursor vaccenic acid (VA), which have potential health benefits, including anticarcinogenic properties in experimental animals (Hughes & Dhiman, 2002).

Milk fat composition can be modulated by supplementing the diets of cows with unsatured lipids to meet consumers' demands. The main sources of polyunsaturated fatty acids (PUFA) are oilseed lipids, such as flaxseed (Glasser et al. 2008), that can be fed to dairy animals to modify the milk fatty acid profile of milk for human consumption (Caroprese et al. 2010). In previous experiments whole flaxseed supplementation of dairy cows at about 7% of daily DM intakes succeeded in increasing PUFA in milk. However, oilseed supplementations, and in particular flaxseed, have a negative impact on management costs of dairy farms, thus discouraging farmers in their utilisation. As a consequence, the study of the reduction of the amount of flaxseed administration in the cows' diet in order to reduce costs and to obtain improvement on milk yield and composition is required.

In Italy, about 80% of dairy farms produce milk from Friesian cows both for direct consumption and for cheese production. In addition, Jersey milk is used to increase cheese yield production and meet market demand in terms of suitability for cheese making. The use of Jersey milk for Cheddar cheese making was found to lead to an improvement in profit for the cheese makers, especially at higher inclusion rates (Bland et al. 2015). However, Friesian and Jersey cows showed differences in the composition of milk fat when increasing dietary intake of calcium salts of palm fatty acid distillate were tested (Beaulieu & Palmquist, 1995).

Milk coagulating properties can be influenced by composition and structural organisation of proteins and fat milk (Logan et al. 2014). Flaxseed administration to lactating ewes, besides changing milk fatty acid composition, succeeded in enhancing milk coagulating properties, probably as a result of increasing fat and casein content (Caroprese

*For correspondence; e-mail: mariangela.caroprese@unifg.it

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et al. 2011). Timmen & Patton (1988) affirm that milk fat globule (MFG) size could be influenced by differences in fatty acids composition. Both MFG and casein micelle (CM) size can be influenced by genetics variations (Bijl et al. 2014; Logan et al. 2014), and feed (Devold et al. 2000; Couvreur & Hurtaud, 2007). Our hypothesis is that flaxseed administration could have an effect on milk coagulation properties, thus influencing its cheese-making ability, by altering the milk fatty acid composition. The objectives of the present experiment were to: (i) evaluate the effects of flaxseed administration on fatty acid profile of milk from Friesian and Jersey cows, and (ii) evaluate the change in milk coagulation properties in Friesian and Jersey cows as affected by flaxseed administration.

Materials and method

Animals, experiment al design, and dietary treatments

The experiment was conducted in a farm located in Gravina in Puglia (BA), Apulia, Southern Italy (latitude: 40°49′14″ 52 N and longitude 16°25′24″96 E). A 30 d trial was performed from May to June of 2014 with 20 Italian Friesian cows and 20 Jersey cows divided into 2 groups of 10 animals which were balanced according to days in milk (112·31 ± 5·15 d). Experimental diets were (1) a conventional diet (CON) administrated as unifeed (2) a diet containing 0·5 kg/d of whole flaxseed (FS) in substitution of an equal amount of cotton seed administrated in the unifeed. Formulation of experimental diets and chemical composition of the diets are shown in Table 1. Cows were housed in tie stalls and individually fed; water was available ad libitum. The diets were fed twice daily and each group of cows was fed separately.

Table 1. Chemical composition, and NE_L of the experimental diets (DM basis)

	Diet	
	CON	FS
Corn	33·22	33·22
Oat hay	27·42	27·42
Wheat straw	4·25	4·25
Wheat middlings	11·59	11·59
Whole cotton seeds	1·93	–
Soybean meal	8·50	8·50
Sunflower meal	3·86	3·86
Additional mixed feed†	7·72	7·72
Flaxseed‡	–	1·93
Minerals§	1·51	1·51
DM, %	95·14	93·11
Ether extract, %DM	3·39	5·01
CP, %DM	14·87	14·90
Ashes	8·43	7·27
ADF, %DM	24·42	23·27
NDF, %DM	37·85	37·56
ADL, %DM	4·40	2·80
NE_L, Mcal/kg¶	1·51	1·51
Fatty acids, % total of fatty acids		
C14 : 0	1·84	1·81
C16 : 0	32·88	32·39
C16 : 1	0·93	0·92
C18 : 0	3·11	3·11
C18 : 1 *cis*-9	19·23	19·10
C18 : 2 *cis*-9, *cis*-12	29·99	29·73
C18 : 3 *n*-3	1·05	2·07
C20 : 5 *n*-3 (DHA)	0·85	0·83
C22 : 6 *n*-3 (EPA)	0·32	0·31

†Sunflower, corn gluten feed, sugar beet pulp.
‡Lin Tech (Tecnozoo srl, Torreselle di Piombino Dese, Italy).
§Ca salts of fish oil, $NaHCO_3$, NaCl.
¶Calculated according to NRC (2001).

Feed sampling and analysis

The chemical composition of diets was determined by standard procedures (AOAC, 1990). The crude fibre and fibre fractions were determined by Fiber Cap (FC 221, FOSS). A representative sample of feed was collected for fatty acid (FA) analysis of feed according to O'Fallon *et al.* (2007). The fatty acid methyl esters (FAME) were analysed on a Agilent 6890N gas chromatograph. Separation of the FAME was performed using a DB 23 fused-silica capillary column 60 m × 0·25 mm (i.d.) with 0·25 µm film thickness. Operating conditions were: helium flow rate of 1·2 ml/min; FID detector at 250 °C; a split-splitless injector at 240 °C and an injection volume of 1 µl with a split ratio 1 : 50. The initial column temperature was set at 60 °C, increased to 180 °C at 25 °C/min and finally increase to 230 °C at 6 °C/min and held for 15 min. Individual FAME's peaks were identified using standards from Matreya (Matreya, 2178 High Tech Road, State College, PA 16803 USA). Each fatty acid was reported as a percentage of FAME (NRC, 2001).

Milk sampling and analysis

Cows were milked twice daily (07 : 00 and 14 : 30) using pipeline milking machines. Milk yield was recorded daily by means of graduated measuring cylinders attached to individual milking units. At the beginning, at day 15 and then at day 30 of the experiment milk samples consisting of proportional volumes of morning and evening milk, were individually collected in 200 ml sterile plastic containers after cleaning and disinfection of teats (70% ethyl alcohol) and discharging the first streams of foremilk. Milk samples were carried in our laboratory by means of transport tankers at 4 °C. One aliquot was stored at −20 °C for fatty acid analysis. Fresh samples were used for chemical analysis as following: pH (GLP 21 Crison, Spain), total protein, casein, fat and lactose content using an infra red spectrophotometer (Milko Scan 133B; Foss Electric, Hillerød, Denmark) according to the International Dairy Federation standard (IDF, 1990); evaluation of the milk coagulation properties (rennet coagulation time, time to

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clot firmness, and clot firmness after 30 min) measured by a Foss Electric Formagraph (Foss Electric, Hillerød, Denmark). At day 30 of the experiment bulk milk from each experimental group was collected and taken to our laboratory by means of transport tankers at 4 °C to perform electrophoresis analysis.

For the analysis of milk fatty acids, milk fat was extracted according to the procedure of Luna et al. (2005) and transesterification of fatty acids according to ISO-IDF (2002) procedures. Briefly, fatty acid methyl esters were separated and measured using a gas chromatograph (Agilent 6890N) equipped with CP-Sil 88 fused-silica capillary column (100 m × 0·25 mm i.d. with 0·25-µm film thickness). Operating conditions were a helium flow rate of 1 ml/min, a flame-ionisation detector at 260 °C, a split-splitless injector at 260 °C, and an injection volume of 1 µl with a split ratio 1 : 50. The temperature program of the column was set at 100 °C with a subsequent increase to 240 °C at 3·5 °C/min and held for 15 min. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards (FIM-FAME-7-Mix, Matreya LLC, Pleasant Gap PA), with the addition of C18 : 1–11t, C18 : 2–9c, 11t, C18 : 2–9c, 11c, C18 : 2–9t, 11t, and C18 : 2–10t, 12c (Matreya LLC, Pleasant Gap PA). Data were collected and integrated using Agilent Chemstation Software rev. B.04·03. Fatty acids were reported as grams per 100 g of FA. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA were calculated. Atherogenic (AI) and thrombogenic (TI) indices were calculated according to Ulbricht and Southgate (1991) formula.

Electrophoresis analysis of caseins

Polyacrilamide gel isoelectric focusing. Casein fraction from bulk milk of Friesian and Jersey cows, fed in CON and FS condition, was prepared according to the procedure of Aschaffenburg & Drewry (1959). Wet caseins were freeze-dried and stored at −20 °C until electrophoresis analysis. Isoelectric focusing was performed on a thin layer polyacrylamide gel (0·25 × 120 × 100 mm^3) in a pH gradient 2·5 ÷ 6·5 obtained by mixing pharmalyte 2·5–5·0, 4·0–6·5 and 5·0–6·0 in the proportion 42, 42 and 16% (v/v), respectively. Electrophoretic conditions were: 1000 Vmax, 4 mA max and 20W for 700 V/h in the pre-focalisation phase and 1500 Vmax, 4 mA max and 20W for 4490 V/h.

Two dimensional gel electrophoresis. Each sample (0·10 g of wet casein) was dissolved in 1·0 ml of 9 M urea to obtain a sample solution. An aliquot of sample solution (100 µl) was mixed with 150 µl of DeStreak and 100 mM DTT to obtain 250 µl of rehydration solution. The immobilised pH linear gradient (IPG) 13-cm dry strips in pH 3–10 (Amersham Biosciences, Melbourne, VIC, Australia) were placed in the 250 µl of rehydration solution, covered with the cover fluid (Amersham Biosciences, Melbourne, VIC, Australia) and kept for a minimum of 8 h at room temperature. Rehydrated IPG strips were submitted to the first dimension using an Ettan IPGphor 3 (GE-Healthcare Bio-Sciences) electrophoresis unit (Amersham Biosciences). Electrophoretic separation was run in 4 steps: 500 V/h followed by 1000 V/h, a gradient to 8000 V over 90 min, and 8000 V for 3 h for a total of 36000 V/h. After the IPG electrophoresis, the strips were equilibrated, first in a solution containing 1·5 M Tris-HCl, pH 6·8, 6 M urea, 30% glycerol (w/v), 2% SDS, 64 mM DTT, and traces of bromophenol blue, for 15 min, and then, in a solution containing 1·5 M Tris-HCl, pH 6·8, 6 M urea, 30% glycerol (w/v), 2% SDS, 135 mM iodoacetamide, and traces of bromophenol blue, for further 15 min. The equilibrated IPG dry strips were loaded on top of the 8–18% SDS-PAGE gradient polyacrylamide gel (1·5 × 18 × 16 mm^3), embedded with 1% agarose. The electrophoresis was carried out in an Hoefer SE 600 Series Ruby Standard Vertical Electrophoresis Unit (GE-Healthcare Bio-Sciences) at a constant voltage of 100 V, at 30 mA/gel, for 16 h at 15 °C. Gels were stained with 0·25% w/v CBB overnight and destained in water. Images were captured on an Image Master Scanner (GE-Healthcare Bio-Sciences) in transmission mode, at a resolution of 300 dpi.

Statistical analysis

Data were processed using ANOVA with the REPEATED statement in PROC MIXED with CV (variance components) as covariance structure of SAS (SAS, 2013). Diet, time of sampling, breed, and their interactions were Fixed factors. Animal was a Random factor nested in the treatment. When significant differences between means were found ($P < 0·05$), a Tukey post-hoc test was performed to adjust tests in multiple comparison.

Results and discussion

Milk yield and milk composition

Average of the three sampling times of the milk yield and composition of cows fed control diet (CON) and flaxseed (FS Table 2) is shown in Table 2. Milk production was influenced only by breed; Friesian cows showed higher milk production than Jersey cows ($P < 0·001$). In previous experiments FS supplementation to Friesian cows increased milk yield (Caroprese et al. 2010, 2013); in the present experiment the absence of apparent effects of FS administration on milk yield, however, was an expected result based on the reduction of FS supplemented by about three-fold. In the present experiment protein and casein yield, and milk composition were significantly influenced only by breed. It is well known that Jersey cows yield lower milk than Friesian cows with higher fat content than milk from Friesian cattle (4·1–4·9 *vs*. 3·3–4·1%, respectively) (Buchanan et al. 2002). Differences in milk yield and composition between Jersey and Friesian milk can be ascribed

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Table 2. Least Square Means ± SEM of milk yield, composition of Friesian and Jersey cows fed control diet or flaxseed (CON and FS)

	Friesian		Jersey			Effects, *P*		
	CON	FS	CON	FS	SEM	Breed	Diet	Breed × Diet
DMI, kg/d	26·06	26·05	26·06	26·04	0·05	NS	NS	NS
Production, kg/d								
Milk yield	16·87a	16·05a	11·06b	11·51b	0·46	***	NS	NS
Fat yield	0·53	0·52	0·52	0·55	0·02	NS	NS	NS
Protein yield	0·58a	0·56a	0·45b	0·47b	0·02	***	NS	NS
Casein yield	0·45a	0·43a	0·35b	0·38b	0·01	***	NS	NS
Milk composition, %								
Fat	3·20b	3·31b	4·79a	4·81a	0·18	***	NS	NS
Protein	3·42b	3·50b	4·07a	4·12a	0·55	***	NS	NS
Casein	2·65b	2·69b	3·14a	3·28a	0·06	***	NS	NS
Lactose	5·01a	4·96ab	4·88b	4·90ab	0·03	**	NS	NS

NS, not significant.
*, $P < 0·05$; **, $P < 0·01$, ***, $P < 0·001$.

both to differences in genetics and to different capability of utilising dietary fibre and N between Jersey and Friesian as reported in Aikman et al. (2008). Those authors reported that despite the faster rate of passage through the gastrointestinal tract, the digestibility of dietary components by Jersey cows was similar or higher than Friesian as a result of increased eating and rumination time per kilogram of DM consumed.

Milk fatty acid composition

Table 3 shows the average of each milk fatty acid at the three sampling times. Milk C10 : 0 and C12 : 0 content was influenced by diet ($P < 0·001$ for C10 : 0 and $P < 0·01$ for C12 : 0,) with higher contents measured in milk from CON than in milk from FS diet. Both C14 : 0 and C16 : 0 milk contents were influenced by breed ($P < 0·05$), and diet ($P < 0·01$) displaying higher contents on average in Jersey than in Friesian milk. These results are in agreement with previous reports (Drackley et al. 2001), in which C16 : 0 content was higher for Jersey milk than for Friesian milk; however, in Drackley et al. (2001) experiment fat supplementation did not influence C16 : 0 content of milk. The content of C18 : 0 was significantly influenced by breed and by the interaction breed x diet ($P < 0·001$ and $P < 0·01$, respectively). The C18 : 0 content was higher in Jersey than in Friesian milk, and in FS milk from Jersey than from Friesian in agreement with previous findings (Mustafa et al. 2003; Loor et al. 2005, Caroprese et al. 2010). C18 : 1*trans*-11 showed higher values in Friesian than in Jersey milk ($P < 0·001$), and in FS than in CON milk ($P < 0·01$). In addition, a significant interaction breed x diet was found, with higher content of C18 : 1*trans*-11 measured in milk from FS Friesian cows than from FS Jersey cows ($P < 0·01$). As reported by Bell et al. (2006) the synthesis of C18 : 1*trans*-11 is influenced by the accumulation of trans C18 : 1 in the rumen. It could be argued that Friesian and Jersey cows are characterised by differences in ruminal biohydrogenation. Whole flaxseed administration, however, in Friesian breed succeeded in enhancing milk content of C18 : 1*trans*-11, which is considered the main health promoting fatty acid in milk together with CLA (Bauman & Lock, 2006). The increase of C18 : 1 isomers can be the result of a partial biohydrogenation in the rumen due to increasing of linolenic acid with flaxseed diet, and of the desaturation of C18 : 0 in the mammary gland (Kennelly, 2006). The evolution of C18 : 3 *n*-3 in milk from Friesian and Jersey cows fed control diet or flaxseed during time is shown in Fig. 1. In general, significant differences were found in C18 : 3 *n*-3 content, which was influenced by breed ($P < 0·01$) and diet ($P < 0·001$). No differences at the beginning and at 15 d were found; whereas, at 30 d of the experiment FS milk from both Friesian and Jersey cows displayed significantly higher contents than CON milk from both Friesian and Jersey cows. On average, the content of C18 : 3 *n*-3 was higher in Friesian milk than in Jersey milk, according to Drackley et al. (2001). However, in the present experiment, flaxseed administration resulted in an enhancement of C18 : 3 *n*-3 content to such an extent that no differences between supplemented Friesian and Jersey cows were found at the end of the experiment. This is in agreement with Palladino et al. (2010).

Among the CLA isomers in milk CLA *cis*-9*trans*-11 was influenced by breed with higher contents in Friesian than in Jersey milk ($P < 0·001$). Nevertheless, even if no effects of diet on milk CLA content was measured, the diet led to an increase in both C18 : 3 *n*-3 and in C18 : 1*trans*-11 in Friesian milk, suggesting that reducing the amount of flaxseed administration can still enrich milk fatty acid profile. In addition, our results confirmed that significant genetic variation exists for the concentration of several fatty acids in milk, and this is most likely a function of differential expression of genes coding for enzymes catalysing FA synthesis within the mammary gland (Soyeurt & Gengler,

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Table 3. Least Square Means ± SEM of fatty acid composition (g/100 g of total fatty acids) of milk from cows fed control diet or flaxseed (CON and FS)

	Friesian		Jersey			Effects, *P*		
	CON	FS	CON	FS	SEM	Breed	Diet	Breed × Diet
C4 : 0	6·52	6·35	6·89	6·82	0·20	*	NS	NS
C6 : 0	2·37b	2·16b	2·97a	3·02a	0·12	***	NS	NS
C8 : 0	1·45b	1·35b	1·88a	1·85a	0·06	***	NS	NS
C10 : 0	3·01b	2·62c	3·95a	3·65a	0·09	***	***	NS
C11 : 0	0·11b	0·12b	0·13a	0·13a	0·01	*	NS	NS
C12 : 0	3·30b	3·00b	4·29a	4·00a	0·10	***	**	NS
C13 : 0	0·13	0·15	0·16	0·16	0·01	NS	NS	NS
C14 : 0	9·99b	9·48b	10·68a	10·28a	0·16	*	**	NS
C16 : 0	25·48b	24·96b	26·96a	25·48b	0·34	**	**	NS
C17 : 0	0·16	0·26	0·22	0·21	0·02	NS	NS	*
C18 : 0	12·64bc	11·74c	13·22ab	14·16a	0·29	***	NS	**
C20 : 0	0·21a	0·20a	0·18a	0·14b	0·01	***	**	NS
C22 : 0	0·13a	0·09b	0·08b	0·10b	0·01	NS	NS	***
C14 : 1	0·22	0·21	0·20	0·23	0·01	NS	NS	*
C14 : 1c	1·04b	1·15a	0·93c	0·99bc	0·02	***	***	NS
C15 : 1 *trans*	0·05b	0·04b	0·13a	0·09ab	0·01	***	*	NS
C16 : 1 *trans* t	0·68b	0·91a	0·64b	0·61b	0·03	***	**	***
C16 : 1*cis*	0·04b	0·05a	0·04b	0·04b	0·00	*	**	NS
C17 : 1*trans*	0·12	0·07	0·06	0·06	0·02	NS	NS	NS
C18 : 1t6	0·00	0·00	0·08	0·10	0·02	***	NS	NS
C18 : 1*trans*-9	0·39	0·41	0·28	0·37	0·03	**	NS	NS
C18 : 1*trans*-11	2·67b	3·80a	1·24c	1·31c	0·15	***	**	**
C18 : 1*cis*-6	0·13a	0·02b	0·05ab	0·06a	0·02	NS	*	*
C18 : 1*cis*-9	21·55a	22·79a	18·29b	19·49b	0·35	***	***	NS
C18 : 1*cis*-11	0·68b	0·82a	0·45d	0·52c	0·02	***	***	NS
C19 : 1*trans*-7	0·17b	0·26a	0·14b	0·17b	0·01	***	***	***
C19 : 1*trans*-10	0·57	0·24	0·33	0·17	0·12	NS	*	NS
C20 : 1*cis*-11	0·05	0·05	0·07	0·06	0·01	NS	NS	NS
C20 : 1*trans*-11	0·18c	0·27a	0·11d	0·23b	0·01	***	***	NS
C18 : 2*trans*-9, *trans*-12	0·09	0·08	0·09	0·08	0·01	NS	**	NS
C18 : 2*cis*-9, *cis*12	4·08	4·38	3·78	3·92	0·16	*	NS	NS
C20 : 2	0·03b	0·05a	0·03b	0·03b	0·02	***	NS	NS
C22 : 2	0·02b	0·03a	0·02c	0·03a	0·0001	***	***	NS
C18 : 3 *n*-3	0·26b	0·31a	0·22c	0·28bc	0·01	**	***	NS
C18 : 3 *n*-6	0·03b	0·02b	0·06b	0·10a	0·01	***	NS	*
C20 : 3 *n*-3	0·29a	0·27a	0·23b	0·23b	0·01	***	NS	NS
C20 : 3 *n*-6	0·12b	0·13b	0·17a	0·12b	0·10	*	NS	**
CLA 9c11t	0·70a	0·79a	0·40b	0·40b	0·03	***	NS	NS
CLA 10*trans*-12*cis*	0·02b	0·04a	0·01c	0·01c	0·001	***	***	***
CLA 11*cis*-13*cis*	0·04a	0·04a	0·02b	0·03b	0·0001	***	NS	NS
CLA 11*cis*-13*trans*	0·004a	0·006a	0·001b	0·003ab	0·0001	***	*	NS
CLA *trans,trans*	0·01	0·01	0·02	0·02	0·0001	*	NS	NS
C20 : 4 *n*6	0·01	0·01	0·01	0·01	0·0001	NS	NS	NS
C22 : 4	0·09a	0·07b	0·06c	0·06c	0·0001	***	***	**
C20 : 5 *n*-3	0·015a	0·015a	0·011b	0·013b	0·0001	***	NS	NS
C22 : 5 *n*-6	0·020a	0·017b	0·015b	0·015b	0·0001	***	NS	NS
C22 : 5 *n*-3	0·05	0·05	0·03	0·04	0·0001	***	**	NS
C22 : 6 *n*-3	0·02	0·02	0·02	0·02	0·0001	*	NS	NS
SFA	65·54c	62·51d	71·75a	70·04b	0·45	***	***	*
MUFA	28·53b	31·05a	23·00d	24·49c	0·40	***	***	*
PUFA	5·93b	6·41a	5·24b	5·46b	0·18	***	NS	NS
*n*6	5·27ab	5·70a	4·69c	4·83bc	0·17	***	NS	NS
*n*3	0·61a	0·65a	0·49b	0·57a	0·01	***	***	NS
A.I.	2·01c	1·80d	2·68a	2·41b	0·06	***	***	NS
T.I.	2·56c	2·29d	3·32a	3·06b	0·04	***	***	NS

NS, not significant.
*, $P < 0·05$; **, $P < 0·01$; ***, $P < 0·001$.

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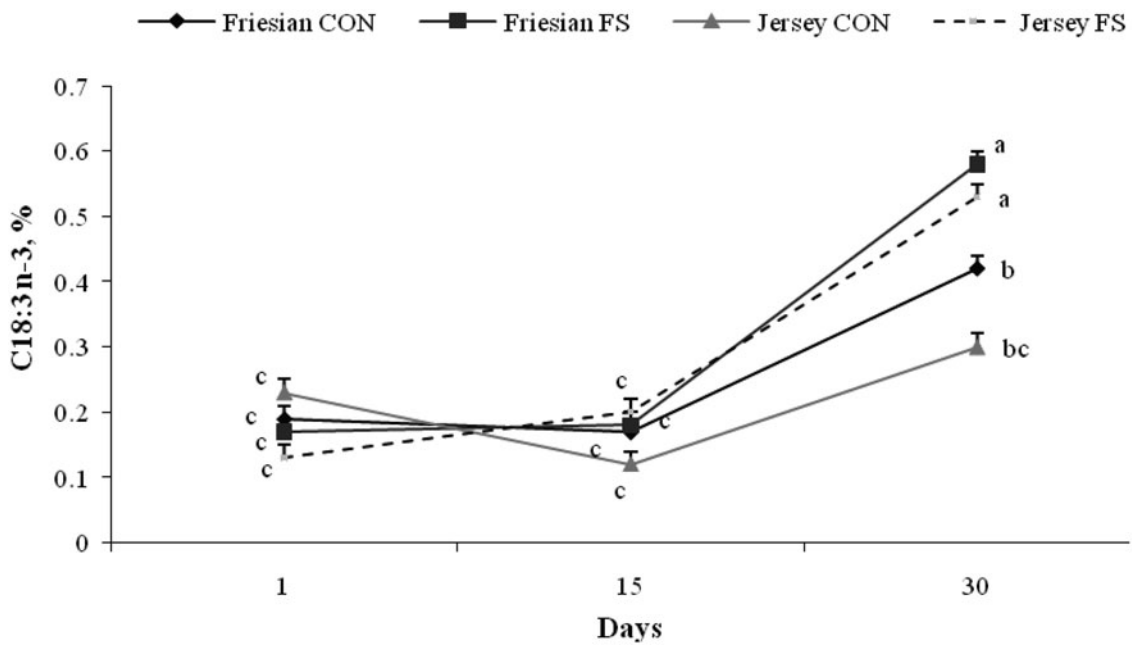

Fig. 1. Least Squares means ± SEM of C18 : 3 *n*-3 content measured in milk from Friesian and Jersey cows fed control diet or flaxseed (CON and FS) at 0, 15, and 30 d of the experiment. [a,b,c] Values with different superscripts differ between feeding treatments within a sampling day ($P < 0{\cdot}05$).

2008; Mele et al. 2009). SFA, MUFA, and PUFA were influenced by breed ($P < 0{\cdot}001$); on average milk from Jersey cows showed higher values of SFA, and lower of MUFA, and PUFA than milk from Friesian cows. In general, flaxseed administration resulted in a reduction of SFA ($P < 0{\cdot}001$) and an increase in MUFA ($P < 0{\cdot}001$) content. Furthermore, an effect of the interaction breed x diet was found for both SFA and MUFA ($P < 0{\cdot}05$); milk from FS group displayed lower SFA and higher MUFA than CON milk both in Friesian and in Jersey milk. Furthermore, the diet supplemented with flaxseed resulted in increased content of *n*-3 FA in milk. The Atherogenic and Trombogenic indexes were influenced by breed ($P < 0{\cdot}001$) and diet ($P < 0{\cdot}001$) with lower values in flaxseed group especially in Friesian cattle. Milk from FS cows was characterised by reduced Atherogenic and Trombogenic indexes, according to previous findings in Caroprese et al. (2010), suggesting that, even at low level of supplementation, flaxseed in the diet of dairy cows yields milk with healthier features from a human perspective.

Milk coagulation properties and Electrophoresis analyses

Milk coagulating properties shown in Fig. 2 were significantly influenced by diet ($P < 0{\cdot}01$ for coagulation time; $P < 0{\cdot}001$ time to clot firmness and clot firmness after 30 min). Jersey cows showed lower values of coagulation time, and time to clot firmness, and higher values of clot firmness at 30 min than Friesian cows. Breed comparison revealed that milk from Jersey cows generally exhibited superior coagulation properties when compared with milk

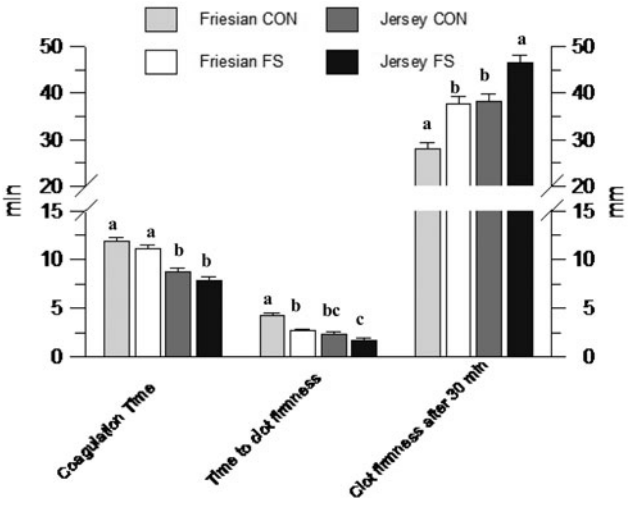

Fig. 2. Least Squares means ± SEM of coagulation time, time to clot firmness, and clot firmness after 30 min measured in milk from Friesian and Jersey cows fed control diet or flaxseed (CON and FS). [a,b,c]Values with different superscripts differ between feeding treatments within a sampling day ($P < 0{\cdot}05$).

from Friesian cows (Jensen et al. 2012). FS diet reduced time to clot firmness and increased clot firmness in Friesian milk; it resulted in increased clot firmness in Jersey milk. In Friesian milk time to clot firmness was affected by the interaction diet x breed, showing a significant reduction in flaxseed when compared to the control group.

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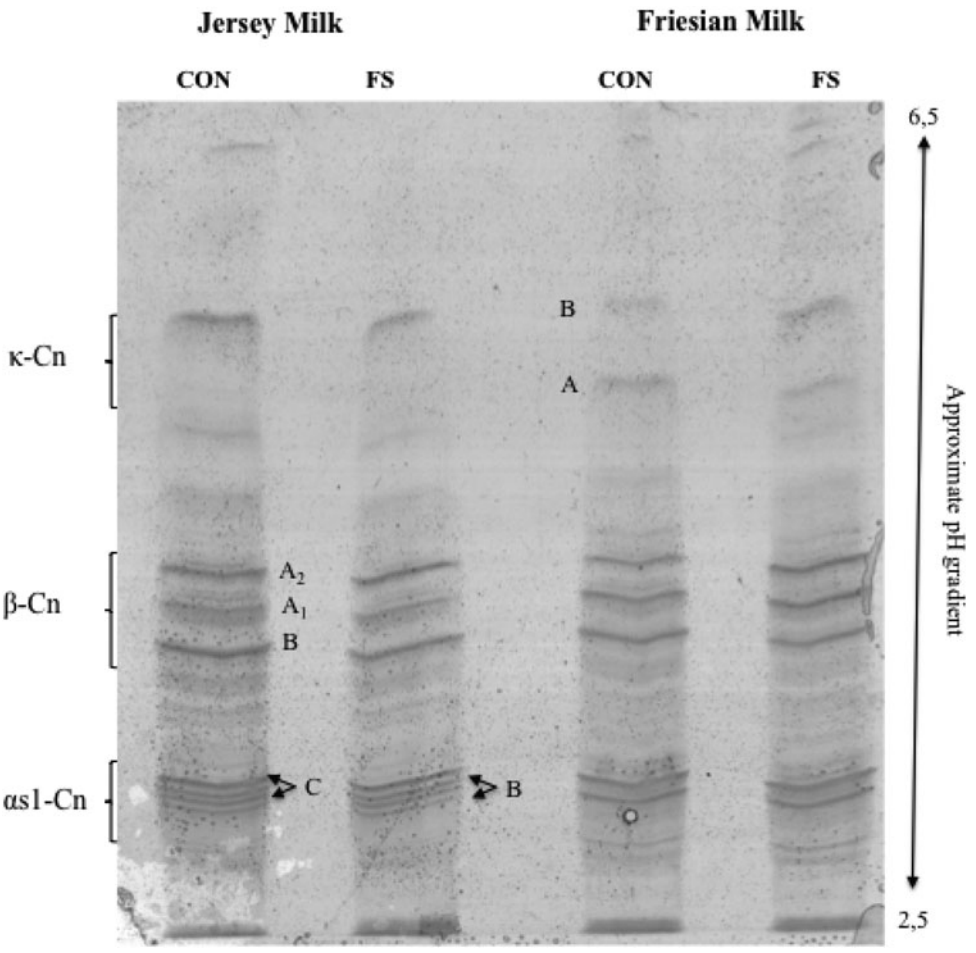

Fig. 3. Polyacrylamide gel isoelectric focusing in the pH range 2·5–6·5 of whole caseins from Friesian and Jersey cows fed control diet or flaxseed (CON and FS). Different genetic polymorphism was revealed in two breed.

To better investigate the results of flaxseed administration on milk coagulating properties and to examine the relation between changes in fatty acid profile and casein fractions from Friesian and Jersey bulk milk, an electrophoresis study was performed. It was stated that cows carrying the α_{s1}-B, β-B, κ-B caseins and β-LG B haplotype show the better coagulation properties (Aleandri et al. 1990; Jakob & Puhan, 1992). Isoelectrofocusing patterns of Jersey and Friesian whole caseins (Fig. 3) showed a different genetic polymorphism. Jersey cows carried homozygous variant B at locus κ-CN, heterozygous variants A1, A2 and B at locus β-CN and heterozygous variants B and C at locus α_{s1}-CN. Friesian cows carried heterozygous variants A and B at locus κ-CN, heterozygous variants A1, A2 and B at locus β-CN and homozygous variant B at locus α_{s1}-CN. Friesian and Jersey milk also differed for the content of variant A1 at β-CN locus as a result of a different distribution of this variant. Beside the different α_{s1}-CN variants found in Friesian and Jersey cows, the 2DE maps showed a different form of the α_{s1}-CN spots in milk from cows fed flaxseed with respect to 2DE maps of milk from control cows irrespective of breed. Previous findings led to the hypothesis that a different aggregation of α_{s1}-CN fraction occurred in bulk milk from flaxseed fed cows (Fig. 4).

The α_{s1}-CN membranous form has been suggested to play a key role in the early steps of casein micelle biogenesis and/or casein transport during the secretory pathway, being required for the efficient export of the other caseins from the endoplasmic reticulum (ER: Chanat et al. 1999; Le Parc et al. 2014). The lipid raft interaction of α_{s1}-CN with the mammary epithelial cell membranes is strengthened by its aggregative properties acting through disulphide bonds (Le Parc et al. 2014). A strong relation between α_{s1}-CN and biophysical properties of milk fat globules (MFG) has been found, in particular, the genetic polymorphism at α_{s1}-CN locus can affect both structure and composition of MFG membrane (MFGM: Cebo et al. 2012). Therefore, a strong relation between fat composition of MFGM and α_{s1}-caein fraction is well documented. In the present study, cows fed flaxseed increased by 6% to about 9% the proportion of unsaturated fatty acids, respectively in Jersey and Friesian milk, compared with control milk. The alteration in the proportion of unsaturated fatty acids induced by flaxseed could have influenced the size and

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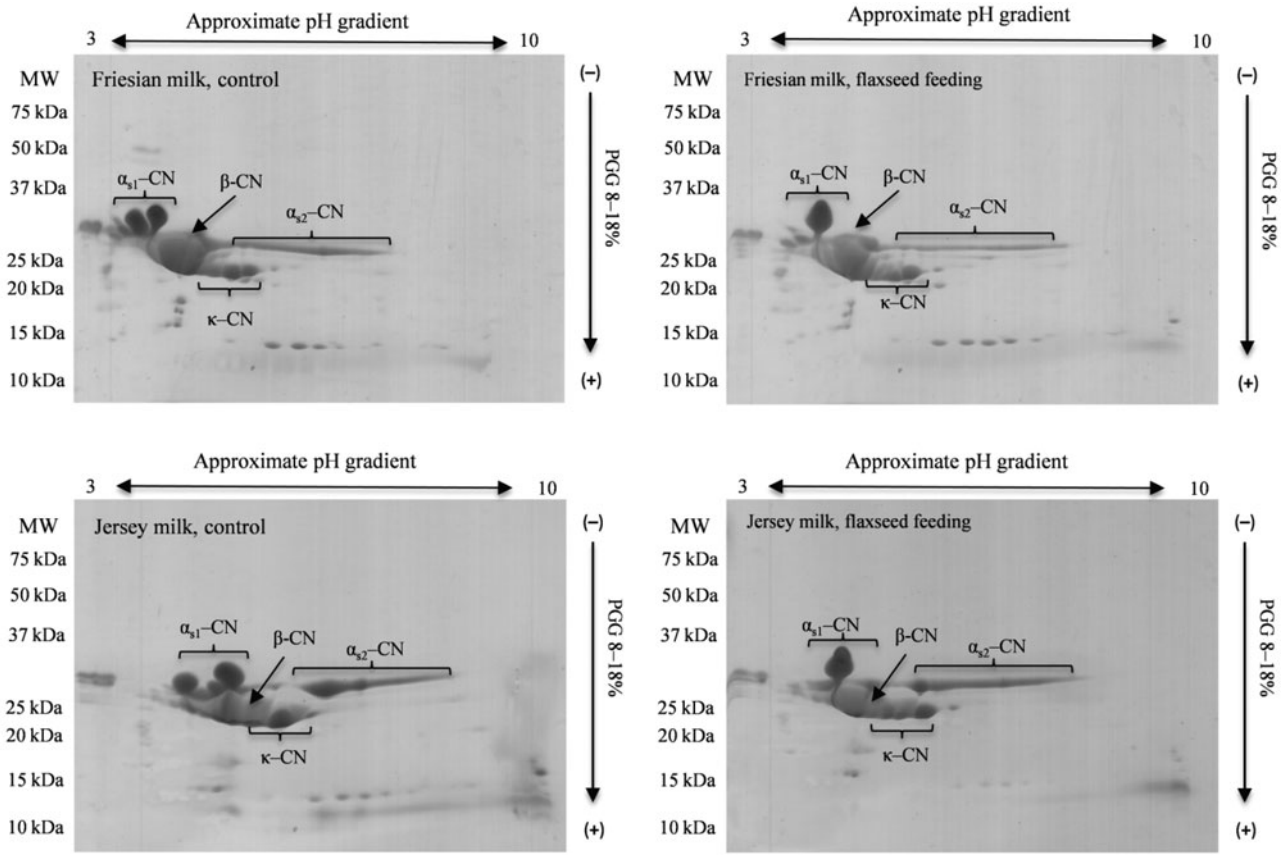

Fig. 4. Two dimensional gel electrophoresis (2-DE) of whole caseins from Friesian and Jersey cows fed control diet or flaxseed (CON and FS). 2-DE maps were obtained by using IPG linear gradient 3·0–10·0 in first dimension and SDS-pore gradient gel 8–18% electrophoresis (PGGE) in second dimension.

behaviour of milk fat globule as previously demonstrated by Briard et al. (2003); Couvreur et al. (2007) and Wiking et al. (2004). Previous studies found that milk from cows fed supplemental fish meal, fish oil and sunflower oil or pasture displayed a decrease in MFG and casein micelles (CN) size (Jones et al. 2000; Avramis et al. 2003; Couvreur et al. 2007). Therefore, the interaction of MFGM-CM influenced coagulation properties of milk (Couvreur et al. 2007; Logan et al. 2014). On the basis of these considerations and of the changes measured in the proportion of unsaturated fatty acids in milk from cows with flaxseed diet we could hypothesise a rearrangement of MFGM, interacting with casein micelles and responsible for self-assembling of two α_{s1}-CN genetic variants. Further studies are needed to clarify this hypothesis by investigating the changes occurring in MFGM composition from cows with a flaxseed diet.

Conclusions

The results of the present study demonstrated that flaxseed in the diet of dairy cows can improve milk fatty acid profile with a reduction of saturated fatty acids, an increase of monounsaturated and polyunsaturated fatty acids, in particular in Friesian milk. Some differences in the content of saturated fatty acids, and monounsaturated fatty acids on milk fatty acid profile after flaxseed administration emerged between Friesian and Jersey cows.

Milk coagulating ability was affected by the flaxseed administration in both breeds, resulting in a reduced time to clot firmness and increased clot firmness, probably as a result of different aggregation of α_{s1}-casein micelles, caused by the change in fatty acid profile of milk fat globule. Further studies are needed to elucidate the relationships between flaxseed administration and altered milk fat globule size and composition, and in milk fat globule interactions with casein micelles.

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