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Effect of Only Pasture on Fatty Acid Composition of Cow Milk and Ciminà Caciocavallo Cheese

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Abstract

To investigate the effect of only pasture or hay and concentrate feedings on milk and cheese fatty acid composition, 20 lactating Italian Red Pied cows were randomly allocated into two groups. Group E was grazed without integration; Group I was fed with hay *ad libitum* and concentrate mixture. A traditional cheese-making technique was used to make Caciocavallo cheese. Milk from only pasture fed cows showed higher percentages of total PUFA, C18:3 n-3, cis-9,trans-11 conjugated linoleic acid and trans-11 C18:1, and a reduced percentage of C16:0 and C18:2 n-6, in some cases more markedly than extensive systems that provide integrations. The fatty acid profile of cheeses largely reflected that of the corresponding raw milk from which cheeses were made. In conclusion, the fatty acid profile of milk and cheese obtained from extensive system confirms the health benefits of dairy products from cows reared at pasture.

Keywords: milk quality, pasture, fatty acids, cows, cheese

1. Introduction

In Italy most of the milk product is used for cheese making. For this reason, cheese quality and composition of milk used for cheese making represent very important factors for the development of the dairy sector. In the last years, consumers have had a growing interest in “typical” foods, products traditional and unique made in a non industrial environment.

Consumers’ perceptions of typical food quality are strongly influenced by several factors, including cultural influences and personal preferences (Santillo et al., 2012); usually consumers associate the typical food to a genuine and healthy product.

Pasta-filata cheeses, which are very popular in Mediterranean countries, include soft/semi-soft and hard or semi-hard varieties which are subjected to considerable aging before being consumed. Caciocavallo is one of the most typical pasta-filata cheeses. It is manufactured in three different areas, the Balkans, Russia and Italy (Piraino, Zotta, Ricciardi, & Parente, 2005).

Ciminà caciocavallo is a cheese that is produced in a small area of low Calabria, around the commune of Ciminà, and is becoming an element of development of a territory. It is produced using raw cow milk (although some farmer adds small amounts of goat milk, about 10%) from both early morning and evening milking.

This cheese is traditionally produced by small farms using local cows, usually Italian Red Pied and Italian Brown, fed using an extensive system production, with animals fed exclusively at pasture without integration, using upland pastures in summer and lowland pastures in winter, according to an artisanal manufacturing procedure related to popular tradition.

Recently, many local producers produce this typical cheese by more intensive farming systems, abandoning the practice of using rationally the pastures of areas at different altitudes, employing more advanced cheese making processes (using stainless steel equipment and commercial starter culture).

The chemical composition of milk, which depends primarily on farming system, plays a crucial role in milk product quality, especially for raw-milk cheese.

In particular, pasture-based feeding systems may portray a healthy image to milk products, in addition to providing specific organoleptic qualities (Coulon, Delacroix-Buchet, Martin, & Pirisi, 2004) which can give the typicality to a product. The healthy image is confirmed by several studies (Chillard et al., 2007; Valvo et al., 2007; Martin, Pomies, Pradel, Verdier-Metz, & Remond, 2009) that report an increase in the unsaturated fraction of fatty acid and conjugated linoleic acid contents in milk and in dairy products derived from grazing systems.

Although there have been numerous studies on effects of pasture in milk or cheese fatty acid composition (Castillo et al., 2006; Ferlay, Martin, Pradel, Coulon, & Chillard, 2006; Ferlay, Rouel, & Lamberet, 2003; Galina, Osnaya, Cuchillo, & Haenlein, 2007; Bonanno et al., 2013), there are few works in which the animals are fed exclusively at pasture without integration.

The present study aimed to evaluate the differences between the traditional farming system normally used for the production of Ciminà Caciocavallo cheese, essentially characterized by an only pasture diet without supplementary integrations, and the more intensive system production on fatty acid composition of Ciminà Caciocavallo cheese and of the milk used to produce it.

2. Materials and Methods

2.1 Experimental Design and Animal Management

The experiment was carried out in a farm located in Calabria (Italy, 38°19’ N, 16°16’ E). The experimental protocol was approved by the University of Reggio Calabria and animals were handled by specialized personnel following the European Union Guidelines (2010/63/ EU Directive)

Twenty lactating Italian Red Pied cows were divided into 2 groups, with 10 animals each, based on similarity in body weight (635±4.6 kg), milk yield (13.50±0.4 kg/d) and fat content in milk (3.67±0.13 g/100g of milk), to ensure that the two groups were similar. Treatments included: intensive (group I), where the cows were kept indoors and fed with hay *ad libitum* and 5 kg of concentrate mixture for all the experimental period (30% barley, 20% corn, 10% oats, 10 % wheat and 30% bean, 2.5 kg in the morning and 2.5 kg in the evening); extensive (group E), where the animals were allowed to graze a natural pasture every day, had free access to water and received no supplementary feed. The animals of the two groups received the respective experimental diet for the whole lactation (10 months).

Milk sampling started from the second month of lactation, from March to May, 2 samples in March, 2 more in April and May, six milk sampling in all for each cow. Milk yield was recorder at each milking and a milk sample of 500 ml was collected from the two milking of the day (250 ml at 5 am and 250 ml at 5 pm) from each cow.

The milk samples were stored at –20 °C until the end of the trial. Then the milk was thawed for 24 h at 4 °C and, for each cow, a bulked sample was obtained by mixing the milk from each of six samplings.

A traditional cheese-making technique was used to make Caciocavallo cheeses from bulk milk samples collected from the two groups, an amount of about 30 l for each group, at the end of the experimental period.

Briefly, raw milk from each batch was heated to 34-38 °C, and liquid commercial kid rennet was added to curdle the milk. After the milk had clotted (after approximately 18 min), the curd was cut to the size of little maize grain. After being removed from the vat, the curd was pressed into14 cm diameter, cylindrical, perforated and placed on tables and ripened for 4-10h, until the pH reaches a value suitable for stretching in hot water (75-95 °C).

Shapes is salted by immersion in brine (20-25 % of salt) for more than 6 hours. Shapes removed from the brine are placed for drying. Seven caciocavallo of Ciminà cheeses were obtained for each group.

Cheeses aged for 30 d were sampled from the form and were immediately vacuum-packaged and stored at −30 °C pending analysis.

The botanical composition of the pasture consisted of Leguminosae (40 % approximately, mainly *Hedysarum coronarium, Vicia sepium, Trifolium pratense*, *Scorpiurus muricata),* Graminaceae (30 % approximately, mainly *Avena fatua, Anthoxanthum odoratum, Lolium perenne)* and of other plants (30 % approximately, mainly *Achillea ptarmica, Arctium minus, Carduus nutans, Cnicus benedictus, Cupularia viscosa, Cardaria draba, Daucus carota, Camelina sativa, Sinapis arvensis, Sisymbrium officinale, Geranium molle, Thymus praecox, Clinopodium nepeta, Stachys sylvatica*).

2.2 Chemical Analysis of Feeds, Milk and Cheese

Samples of the experimental diets, collected during the trial, were analysed for neutral detergent fibre (NDF) according to Van Soest, Robertson, & Lewis, (1991). Furthermore, according to Association of Official Analytical Chemists (AOAC) standards (1995), feedstuffs were also analysed for ash, crude protein and crude fat (ether extract). The fatty acid composition of the feedstuffs was analysed by gas-chromatography using the method described by Sukhija and Palmquist (1988). Fatty acids (FA) were expressed as g/100 g of total fatty acid methyl esters (FAMEs).

Milk was analyzed for moisture by oven drying at 102±2 °C, fat, protein and lactose contents by mid-infrared spectrophotometry (Milko Scan 134 A/B).

Milk fatty acids were determined as fatty acid methyl esters by gas chromatography. For milk fatty acids analysis, 200–400 mg of fat was collected by centrifuging 10 ml of milk at 2000 × g for 15 min. Milk fatty acid composition was measured according to the modified procedure of Sukhija and Palmquist (1988) as described by Tice, Eastridge and Firkins (1994) and FAME were separated on a CP-Sil 88 capillary column (100 m × 0.25 mm × 0.25 m film thickness) in a Varian CP 3900 gas chromatograph.

Operating conditions were: a helium flow rate of 0.7 mL/min, an FID detector at 260 °C, a split-splitless injector at 220 °C with an injection rate of 120 mL/min, an injection volume of 1 μL. The temperature programme of the column was: 4 min at 140 °C and a sub-sequent increase to 220 °C at 4 °C/min. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The internal standard used was C23:0. A standard fatty acid mixture containing 37 fatty acids and purified known individual fatty acids (FAME mix 37 components from Supelco Inc., Bellefont, PA, USA) were used to provide standard retention times. Fatty acids were identified by comparing with the retention times of fatty acids in standard samples. Fatty acids were expressed as g per 100 g of total fatty acid methyl esters.

For each cheese sample, about 150 g was taken and was used to determine chemical composition and to extract fat for FA profile. Cheese samples were analyzed for moisture by oven at 100 °C (method 926.08; AOAC, 2003), fat by the Mojonnier method (method 933.05; AOAC, 2003), total protein by Kjeldahl (method 2001.14; AOAC, 2002), ash using a muffle furnace at 550°C (method 935.42; AOAC, 2000), cholesterol according to Naeemi, Nissar, Tahani and Montaha (1995).

Extraction of fat from cheese samples was carried out as described by Romano et al. (2011). The FA profile of cheese was determined as previously described for milk.

2.3 Statistical Analysis

Data of milk and cheese fatty acid composition were analyzed according to a complete randomized design by GLM procedure of Minitab software (Minitab Inc., State College, PA, USA). The statistical model included dietary treatment as main factor and animal as random factor. Tukey's test was used for comparing mean values.

3. Results

The chemical composition and the fatty acid profile of the food offered to cows are reported in Table 1. Pasture contained 118 g CP per kg and 486 g NDF per kg. Pasture contained higher proportions of PUFA than foods offered to concentrate-fed cows. In particular, it presented a higher proportion of linolenic acid (C18:3 n-3). On the contrary, concentrate was richer in linoleic acid (C18:2 n-6) than other food.

Table 1. Chemical composition (g/100g dry matter) and fatty acid profile (weight % of total fatty acid methyl esters) of feed offered

	Pasture	Concentrate
Chemical composition		
Dry matter (g/kg wet weight)	210.2	872.8
Crude protein	11.8	14.2
Ether extract	4.0	3.9
Ash	8.9	10.0
NDF[a]	48.6	35.9
Main fatty acids		
C16:0	17.73	23.52
C18:0	2.51	5.27
C18:1 n-9	3.22	14.12
C18:2 n-6	13.66	32.76
C18:3 n-3	59.26	22.08

All the analyses were performed in triplicates; [a]Neutral detergent fibre.

The chemical composition and fatty acid composition of milk samples are reported in Table 2 and in Table 3 respectively. Milk fat content was lower ($P<0.01$) for grazing cows at 3.44 g/100 g milk compared to 3.98 g/100 g milk for concentrate fed cows.

Table 2. Effect of cow feeding system on milk yield and chemical composition (g/100g milk) of milk

	treatment			
	E	I	SEM[a]	*P* value
Milk yield (L cow^{-1} day^{-1})	12.94	14.59	0.301	**
Chemical composition				
Dry matter (g/100g wet weight)	123.2	126.6	0.300	NS
Crude protein	3.21	3.23	0.013	NS
Fat	3.44	3.98	0.022	**
Ash	0.75	0.73	0.004	NS
Lactose	4.94	4.95	0.006	NS

[a] standard error of the mean; *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS. not significant; I, group of cows fed with hay *ad libitum* and concentrate; E, group of cows fed with a natural pasture without supplementary feed.

Table 3. Effect of cow feeding system on milk fatty acid composition (g per 100 g of total fatty acid methyl esters)

	Treatment			
	E	I	SEM[a]	*P* value
C4:0	1.94	2.27	0.0574	NS
C6:0	1.75	1.71	0.0184	NS
C8:0	1.01	1.05	0.0105	NS
C10:0	1.75	2.04	0.0797	*
C12:0	1.83	2.61	0.0791	***
C14:0	8.32	11.47	0.3	***
C14:1 cis 9	1.31	0.87	0.0463	***
C15:0	0.99	1.14	0.0155	NS
C15:1	0.32	0.29	0.0182	NS
C16:0	28.42	30.06	0.237	***
C16:1 cis 9	1.55	0.16	0.131	***
C17:0	0.53	0.61	0.0158	**
C17:1	0.2	0.23	0.006	**
C18:0	13.07	10.21	0.274	***
C18:1 trans 9	0.62	0.65	0.017	NS
C18:1 trans 11	3.50	1.07	0.236	***
C18:1 cis 9	24.49	25.95	0.195	***
C18:2 trans 9 trans 12	0.33	0.35	0.021	NS
C18:2 cis 9 trans 12	0.13	0.08	0.006	***
C18:2 cis 9 cis 12	2.69	3.63	0.132	***
C18:3 cis 6 cis 9 cis 12	0.03	0.04	0.003	NS
C18:3 cis 9 cis 12 cis 15	2.81	1.10	0.171	***
C18.2 cis 9 trans 11	1.97	0.66	0.131	***
∑ STA	51.33	53.49	0.336	**
∑ MUFA	31.99	29.15	0.274	**
∑ PUFA	7.96	5.86	0.096	***
n-6/n-3	0.96	3.30	0.138	***

[a] standard error of means; NS. not significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$. STA. saturated fatty acids; MUFA. monounsaturated fatty acids; PUFA. polyunsaturated fatty acids.

Table 4. Effect of cow feeding system on chemical composition (g/100g cheese) of cheese after 30 days of ripening

	Treatment			
	E	I	SEM[a]	*P* value
Cheese yield value (kg/100 kg of milk)	11.41	12.37	0.294	NS
Chemical composition				
Dry matter (g/kg wet weight)	564.0	553.4	5.23	NS
Crude protein	18.1	17.8	0.213	NS
Fat	12.2	19.9	0.425	***
Ash	4.1	3.7	0.113	NS
Cholesterol (mg/100g)	88.7	101.7	2.25	***

[a]SEM, standard error of the mean; *P<0.05; **P<0.01; ***P<0.001.

Table 5. Effect of cow feeding system on fatty acid composition (g per 100 g of total fatty acid methyl esters) of cheese after 30 days of ripening saturated fatty acids) when the animals were fed with concentrates, while the concentrations of MUFA and PUFA were higher (P<0.01 and P<0.001 respectively) when the cows were fed at pasture. Stearic, myristoleic, palmitoleic and vaccenic acids were higher (P<0.001 for all fatty acids) in cheese from cows that were allowed to graze a natural pasture, such as in milk. Even the two essential fatty acids, as well as the CLA, showed the same trend observed in milk

	Treatment			
	E	I	SEM[a]	significance
C4:0	2.11	2.56	0.0637	NS
C6:0	1.85	1.84	0.026	NS
C8:0	1.1	1.15	0.0201	NS
C10:0	1.95	2.32	0.0477	*
C12:0	1.94	2.8	0.085	***
C14:0	8.59	11.61	0.29	***
C14:1 *cis 9*	1.12	0.78	0.0499	***
C15:0	1.1	1.28	0.0246	NS
C15:1	0.24	0.19	0.0266	NS
C16:0	29.79	31.6	0.25	***
C16:1 *cis 9*	1.25	0.08	0.11	***
C17:0	0.65	0.68	0.0206	NS
C17:1	0.11	0.11	0.0101	NS
C18:0	13.55	10.48	0.311	***
C18:1 *trans 9*	0.46	0.46	0.0195	NS
C18:1 *trans 11*	3.14	0.91	0.229	***
C18:1 *cis 9*	23.15	24.97	0.231	***
C18:2 *trans 9 trans 12*	0.27	0.18	0.0195	*
C18:2 *cis 9 trans 12*	0.13	0.03	0.015	***
C18:2 *cis 9 cis 12*	2.24	2.87	0.043	***
C18:3 *cis 6 cis 9 cis 12*	0.01	0.02	0.0031	NS
C18:3 *cis 9 cis 12 cis 15*	2.07	0.60	0.052	***
C18.2 *cis 9 trans 11*	1.88	0.51	0.137	***
∑ STA	53.68	55.65	0.336	**
∑ MUFA	29.47	27.50	0.285	**
∑ PUFA	6.60	4.21	0.398	***
n-6/n-3	1.08	4.78	0.138	***

[a] standard error of means; NS. not significant; *P<0.05; **P<0.01; ***P<0.001. STA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Milk from grass-fed cows contained lower (P<0.05) levels of SFA and higher levels of both monounsaturated fatty acids (MUFA) (P<0.01) and PUFA (P<0.01) than cows given concentrates. Among saturated fatty acids, capric (C10:0), lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids were higher in milk from cows given concentrates (P<0.05 for C10:0 and P<0.001 for the other three saturated fatty acids), while stearic acid (C18:0) was higher (P<0.001) in milk from cows that were allowed to graze a natural pasture. On the contrary, myristoleic (C14:1) and palmitoleic (C16:1) acids were higher (P<0.01 and P<0.001 respectively) in milk from grass-fed animals compared with those given concentrates. Trans-vaccenic acid was significantly higher (P<0.001) in milk from grass-fed cows compared with animals given concentrates. Linoleic acid (C18:2 n-6) was higher (P<0.01) in milk from cows given concentrates, while linolenic acid (C18:3 n-3) was significantly higher (P<0.001) in milk from grass-fed animals. Conjugated linoleic acid (9 cis, 11 trans C18:2; CLA) was almost triple in milk from grass-fed cows compared with animals given concentrates (P<0.001).

Effect of cow feeding system on chemical composition and fatty acid composition of cheese are reported in Table 4 and in Table 5 respectively. Cheese fat and cholesterol contents were lower for grazing cows at 12.2 g/100 g cheese and 88.7 mg/100 g cheese, compared to 19.9 g/100 g cheese and 101.7 mg/100 g cheese for indoor fed cows, respectively.

Fatty acid profile of cheeses was generally similar to those of milk, suggesting that processing of milk into cheese did not alter its fatty acid composition. In cheese, as in milk, the concentrations of total SFA and of the most important saturated fatty acids as lauric, myristic and palmitic acids were higher (P<0.01 for total SFA and P<0.001 for the three.

4. Discussion

Several studies have reported as high levels of long chain n-6 PUFA, that derived from linoleic acid, are related to cardiovascular diseases and inflammatory disorder, following their metabolization in certain prostaglandins and thromboxanes (Banskalieva Sahlu, & Goetsch, 2000), whereas long chain n-3 PUFA, that derived from α-linolenic acid, have a wide range of biological effects, which are believed to be beneficial for human health (Barlow, Young, & Duthie, 1990; Kromhout, 1989). For this reason the n-6/n-3 ratio is considered a indicator of the nutritional quality of the lipids in a food and values <4 are recommended (Department of Health, 1994; Enser & Wood, 1997; Wood et al., 2004)

In this study fatty acid profile of cheeses was generally similar to those of milk, suggesting that processing of milk into cheese did not alter its fatty acid composition. The proportions of PUFA were higher (P<0.001) in cheese and milk from pasture-fed cows. The levels of PUFA observed in the cheeses from pasture fed cows of the present trial were higher than those reported by Bonanno et al. (2013) and by Esposito et al. (2014). Unlike cows of our trial, the animals of the trials mentioned above received supplementations, or just hay as in the trial of Bonanno et al. (2013) or hay and concentrate as in the trial of Esposito et al. (2014).

Different levels of PUFA in the products from cows of the two groups were mainly due to the level of α-linolenic acid (C18:3 n-3) that was three fold higher (P<0.001) in milk from cows that were allowed to graze a natural pasture than in milk from cows fed concentrates. This is because grass has a higher concentration of α-linolenic acid, mainly in young pasture (Chilliard, Ferlay, & Doreau, 2001). Also for C18:3 n-3 the levels observed in the cheeses from pasture fed cows in present trial were higher than those reported from other authors (Bonanno et al., 2013; Esposito et al., 2014). Probably the absence of supplementations and the stage of physiological maturity of pasture could have influenced these differences.

On the contrary, the level of linoleic acid, present in higher amounts in concentrates, was higher (P<0.001) in milk and cheese from concentrates fed cows. Following the higher levels of α-linolenic acid and the lower levels of linoleic acid in milk and cheese from cows at pasture, the n-6/n-3 ratio was significantly lower in products from this group, below the level indicated by COMA (Department of Health, 1994).

The levels of saturated fatty acids C12:0, C14:0 and C16:0, that are thought to be involved in increasing plasma total and LDL cholesterol (Moloney et al., 2001), were lower in cheese and milk derived from cows that were allowed to graze a natural pasture. The decrease in the proportion of medium chain fatty acids such as C10:0, C12:0 and C14:0 with pasture is in agreement with other authors (Ferlay et al., 2006). This decrease is probably due in part to the more important amount of dietary unsaturated fatty acids as linolenic acid provided by pasture. These polyunsaturated fatty acids, their biohydrogenation products, or both are potent inhibitors of mammary synthesis of fatty acids with 10 to 14 carbons (Bauman & Griinari, 2003).

The difference in palmitic acid content between groups could be attributed to the different level of this fatty acid in the diets consumed by cows. Generally, experimental data on the effect of different diets on the level of C16:0

showed lower levels of this fatty acid in cheese and milk from animals given fresh grass (Valvo et al., 2005; Bonanno et al., 2013). Other studies have shown that cheese from grazing ruminants had better non-saturated fatty acid profiles (Burdank, 2001; Turner, McClure, Weiss, Borton, & Foster, 2002).

With respect to health-promoting FA, pasture feeding increased trans-11 18:1 and cis-9, trans-11 18:2 percentages in milk and cheese. Several studies have investigated possible feeding strategies to increase PUFA and rumenic acid content in meat and milk, via the manipulation of ruminal. From the biohydrogenation of linoleic and linolenic acids, a large number of trans C18:1 isomers are derived and accumulated in tissues (Mosley, Powell, Riley, & Jenkins, 2002; Bessa et al., 2007). According to Griinari et al. (2000) and Sackmann et al. (2003), the most effective way to enhance the concentration of rumenic acid in ruminant products is to favor the ruminal production of vaccenic acid. In the mammary gland (Griinari et al., 2000), vaccenic acid is partially converted to rumenic acid by the action of Δ^9-desaturase enzyme. Some studies have shown that up to the 90 % of the rumenic acid in milk originates through endogenous biosynthesis in the mammary gland (Piperova et al., 2002). The higher 18:3 n-3 intake with grass and the effects of fresh grass components (e.g., sugars) could interact to enhance the ruminal formation of biohydrogenation intermediates of polyunsaturated fatty acids as cis-9 trans-11 18:2 and trans-11 18:1. The levels observed of these fatty acids in the cheeses and in milk from the cows fed at pasture of the present trial were higher than those reported by other author (Ferlay et al., 2006; Bonanno et al., 2013, Esposito et al., 2014), where, as mentioned above, the animals, unlike cows of our trial, received supplementations.

Regarding the chemical characteristics of milk and cheese, in this trial we observed lower levels of fat in milk and cheese ($P<0.01$ and $P<0.001$ respectively) and of cholesterol in cheese from grazing cows. The reduction of fat levels could be explained by the inhibiting effect of high concentrations of PUFA in the diet on the de novo synthesis of fatty acids. Currently there are several recognized mechanisms that could explain this effect (Palmquist, Beaulieu, & Barbano, 1993; Loor & Herbein, 2003).

5. Conclusions

The fatty acid profile of cheese obtained from extensive system demonstrated the health benefits of dairy products from cows reared at pasture. In fact, only pastures feeding increases the proportions of PUFA and α-linolenic acid. Moreover, the levels observed of these fatty acids in the cheeses from pasture fed cows were higher than those reported from other author, where, unlike cows of our trial, the animals received supplementations. Also the contents of trans-11 C18:1, CLAs, which have been ascribed some potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and antiadipogenic, were higher in milk and cheese from pasture fed cows. Moreover, pasture growing reduced n-6/n-3 ratio, a value that showed nutritional importance in the human diet for its anticarcinogenic properties.

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