EFFECT OF FEEDING SYSTEM ON UNSATURATED FATTY ACID LEVEL IN MILK OF DAIRY COWS

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ABSTRACT

In recent years, consumer attitudes toward fat of animal origin have changed owing to findings that some milk fatty acids (FAs) are positive for human health, especially conjugated linolenic acid and *n*-3 FAs. Accordingly, the manipulation of the fat content and FA composition of cows' milk via nutritional strategies has been an important target for the dairy industry in many countries. Twenty commercial Holstein–Friesian dairy herds of Asturias (northern Spain) with 1106 dairy cows were examined in order to evaluate milk FA profiles under different management systems. These herds were divided into three groups according to management: (1) indoor herds: cows feeding indoors, (2) mixed herds: indoor management system but with at least 6 h of grazing outdoors and (3) outdoor herds: cows allowed 6–18 h of grazing per day. Milk from the indoor herds exhibited the highest concentration of fat (3.57%; *P*O0.01), protein (3.14%; *P*O0.001), lactose (4.76%; *P*O0.01) and urea (29.4 mg dl^{-1}; *P*O0.01). The milk of outdoor herds had a lower (*P*O0.05) content of short-chain FAs than that of the indoor and mixed herds (10.89 versus 11.52 and 11.35 g 100 g^{-1} FA). The milk of the indoor herds had higher concentrations of saturated fatty acids (SFA) (67.56 g 100 g^{-1} FA; *P*O0.001) and palmitic and palmitoleic acids (30.16 and 1.82 g 100 g^{-1} FA, respectively), while that of the mixed and outdoors herds had higher concentrations of unsaturated fatty acids (UFA) (34.58 g 100 g^{-1} FA; *P*O0.001) and long-chain FAs, especially stearic (13.89 g 100 g^{-1} FA; *P*O0.01), vaccenic (2.77 g 100 g^{-1} FA; *P*O0.001), conjugated linoleic (0.92 g 100 g^{-1} FA; *P*O0.001) and linolenic (0.42 g 100 g^{-1} FA; *P*O0.001) acids. Results from this study suggest that the incorporation of forage and pasture in the diet of dairy cows can improve the FA profile of milk.

Keywords: pasture, dairy cow, milk quality, fatty acids

INTRODUCTION

Since the entry of Spain into the European Union in 1986 and the adoption of the milk quota system, the country has seen a large reduction in the number of dairy farms. Between 2001 and 2008, there has been a 55% reduction in the number of dairy farms in Spain, from 53,224 to 24,133. This trend in dairy production along the Atlantic Coast of Spain, which accounts for 79% of the milk quota holders, has followed the national trend[1]. While there has been a dramatic reduction in the number of milk quota owners, accompanied by a limited reduction in the number of cows milked, average milk production of remaining farms has experienced a large increase. Genetically improved dairy cattle usually make up the herds of these remaining pro- ducers, who have increased the percentage of concentrates in the feed[2].

Since the cost of inputs (labor, cows, feed, etc.) has increased, but the price of milk and milk products has remained steady or even declined, farmers need to become more efficient to remain profitable. Grazing has been pro- posed as an essential strategy for the efficient use of pastoral resources which are abundant in damp, temperate areas. Northern Spain has a marine west coast climate, generally warm and wet summers, and fairly mild winters. Annual rainfall is above 900 mm and the mean annual temperature is approximately 13ºC. These climatic con- ditions allow annual grazing, which have been used trad- itionally across Atlantic Europe.

Milk contains a high proportion of saturated fatty acids (SFA; 70–75%), which has made it target of criticism by diet and nutrition experts[3]. However, about 2% of milk fatty acids (FA) are polyunsaturated fatty acids (PUFA), including *n*-3 FAs, rumenic and vaccenic acids[4], which have health benefits for consumers[5]. Increasing the concentration of desirable FAs in ruminant products has received greater attention recently[6]. Fresh grass contains a high proportion of its total FA content in the form of C18:3. Cows grazing fresh grass produce milk with improved acid profiles for human health, as shown by a higher proportion of PUFA, especially conjugated linoleic acid (CLA) and linolenic acid[7,8]. Moreover, pasture feeding imparts agree- able sensorial qualities to milk: grassy flavors can be easily recognized by some consumers[9]. Accordingly, feeding fresh forage extends the role of grazing as a natural and sus- tainable production system with potential health benefits.

The aim of this study was to examine milk characteristics among dairy herds along the Atlantic Coast of Spain (Asturias). Information from the study sought to elucidate differences in FA profiles of milk fat from representative feeding methods, thereby identifying potential value-added attributes of milk.

MATERIALS AND METHODS

The milk of 20 commercial Holstein–Friesian dairy herds from northern Spain was examined in spring of 2008. All herds were enrolled in a routine dairy control program (run by Asturiana de Control Lechero, S. Coop. L.) consisting of regular monitoring of milk yield and quality, and cow reproduction and health (Table 1). The total number of dairy cows making up these herds was 1106, approximately 1.25% of all dairy cows in Asturias.

All farms prepared the total mixed ration (TMR) in a mixer-wagon. The selected herds were divided into three groups according to their management: (1) Indoor herds (n = 10): representing the conventional production and feeding system now used in the Atlantic arc. These herds were fed TMR with grass and maize silages plus cereals at a concentrate : forage ratio of 1 : 1. (2) Mixed herds (n = 4): representing a system with low use of preserved forage and a high input of concentrates and by-products. The cows of these farms grazed for up to 6 h per day. (3) Outdoor herds (n = 6): representing a low-input system. These cows grazed between 6 and 18 h per day. Low levels of concen- trate were included in the TMR in these herds. Obtaining additional representative farms using grazing and mixed feeding regimes was difficult due to an unwillingness of local producers to be involved in the study. Consequently, the numbers of herds in each treatment was unbalanced.

Milk bulk tanks were sampled weekly from April to June 2008. Milk samples were taken from the bulk tank after stirring following at least two milkings at each participating farm. One sample of freshly made TMR was taken fortnightly from April to June along the feeders from each farm. Before the cattle moved to each new plot for grazing, the farmers sampled the pasture of this new plot by cutting 1 kg of the grass with a hand mower to a height of 6 cm from four different areas chosen at random. Rotational grazing management resulted in a change of plot every 2–3 weeks, allowing a total of 46 samples of grass. All samples were kept refrigerated and analyzed immediately upon arrival at the laboratory.

All feed samples were dried (60ºC for 24 h) and ground through a 0.75-mm mesh. The nutritive parameters were determined by near infrared spectroscopy (FOSS NIRSys- tem 5000, FOSS NIRSystems, Inc., Laurel, MD, USA). Milk samples were analyzed for fat, protein, urea and lactose contents by Fourier Transform Infrared in accor- dance with AOAC standards (MilkoScan FT 6000, FOSS NIRSystems, Inc.).

The FA content of the feeds was analyzed according to Sukhija and Palmquist[10] using modifications of Palmquist and Jenkins[11]. For milk FA analysis, fat was isolated as de- scribed by Feng et al.[12] and transesterified as described by Christie[13] with modifications of Chouinard et al.[14]. FA methyl esters were determined by gas–liquid chromato- graphy (Varian 4000 GC/MS, Varian Inc., Palo Alto, CA, USA) in both milk and feed samples. Methyl esters were separated using a 100 m*r*0.25 mm i.d. fused silica capi- llary column (CP-Sil 88 Varian). Individual FA peaks were identified by comparison of their retention times and mass spectra with those of pure methyl ester standards (Matreya Inc., PA, USA and Sigma-Aldrich Inc., MO, USA).

Feedstuff and milk data collected over 12 weeks were analyzed using a GLM procedure for unbalanced ANOVA. The statistical analysis was preformed using the SAS statistical package[15] according to the model:

$$Y_{ijk} = \mathrm{m} + M_i + W_j + H_k + E_{ijk},$$

where Y_{ijk} is the dependent variable, m is the overall mean, M_i is the effect of management system, W_j is the week of sampling, H_k is the effect of the herd and E_{ijk} is the residual error. Significant differences were accepted if *P*O0.05.

RESULTS

Chemical composition of feedstuffs and pasture

No differences were observed between farms in terms of the dry matter, organic matter, neutral detergent fiber, starch or net energy contents of TMR (Table 2). The TMR for indoor herds had higher crude protein and crude fat contents and a lower acid detergent fiber content than the TMR for the mixed and outdoor herds. Nearly 90% of the FA of the feedstuffs was composed of palmitic (C16:0), oleic (C18:1 *cis*9) and linoleic (C18:2 *cis*9 *cis*12) acids. The TMR given to the mixed herds had higher concentrations of caprylic (C8:0), capric (C10:0) and myristic (C14:0) acids than that given to the indoor and outdoor herds, and had a lower C16:1 content than outdoor herd TMR. The outdoor herds' feed showed a higher proportion of linoleic and lower stearic (C18:0) and oleic acid contents than those of the mixed and indoor herds. The chemical composition of the pasture was similar between mixed and outdoor systems, and was typical for the region during a wet spring[16]. The predominant FAs in pasture were linolenic (C18:3 *cis*9 *cis*12 *cis*15), palmitic and linoleic acids, which comprised nearly 90% of total FA measured.

Milk composition and FAs profile

Indoor herds showed the highest concentration of protein, fat, lactose and urea (Table 3). The indoor and mixed herds produced milk with a higher content of short-chain FAs compared to the outdoor herds (11.52 and 11.35 versus 10.89 g 100 g^{-1} FA, respectively; *P*O0.05). Saturated medium-chain FA concentration of the indoor and mixed herd milk was higher than that of outdoor herds. Indoor herd milk had lower concentrations of heptadecanoic (C17:0) and heptadecenoic (C17:1) acids and higher concentration of palmitoleic (C16:1) acid than that of the mixed or outdoor herds. In addition, the total medium-chain FA concentration of the indoor herd milk was higher (45.38 g 100 g^{-1} FA, *P*O0.001) than that of the mixed and outdoor herd milk (42.63 and 43.26 g 100 g^{-1} FA for mixed and outdoor herds, respectively). The concentrations of oleic and linoleic acids were not affected by the manage- ment system. The indoor herd milk showed lower con- centrations of stearic, vaccenic (C18:1 *trans*11), rumenic (C18:2 *cis*9 *trans*11) and linolenic acids than that of the outdoor and mixed herds. In addition, the total long-chain FA content of the indoor herd milk was lower (*P*O0.001) than that of the mixed and outdoor herd milk (43.09, 46.00 and 45.83 g 100 g^{-1} FA, respectively).

The indoor herd milk had a higher (*P*O0.001) content of SFA (65.52 g SFA 100 g^{-1} FA) than the mixed and outdoor herd milk (65.25 and 65.47 g SFA 100 g^{-1} FA, respec- tively). The SFA : UFA ratio of the indoor herd milk was significantly greater (*P*O0.001) than that of the mixed and outdoor herd milk (2.09 versus 1.91 and 1.89, respectively). The desaturase activity of the mammary gland[17], estimated for pairs of FAs C14:1–C14:0, C16:1–C16:0 and C18:1– C18:0 was similar for all herd management systems. How- ever, the CLA : C18:1 *trans*-11 ratio was higher (*P*O0.01) for the outdoor and mixed (0.32) herds than for the indoor herds (0.20).

DISCUSSION

Lower dry matter intake and milk production are typically associated with grazing relative to more intensive feeding systems[18]. Certainly, in the present work, milk yield per lactation was higher under the indoor system compared to the other systems, while FA profiles of the herds under the three management systems differed. Fresh forage fed to the mixed and outdoor herds was the main factor behind these differences. The diet of the indoor herds explains the higher short- and medium-chain FA content of their milk[8,19]. This diet could change ruminal fermentation towards propionate production because of a higher proportion of cereal feedstock[20]. Acetate and b-hydroxybutyrate are necessary for the *de novo* synthesis of FAs in the mammary gland[21]. Variations in the milk concentration of short- and medium- chain FAs have been reported, although some authors report no differences in the milk produced by herds under grazing and intensive management systems[22]. The low the mixed and outdoor herds might be a consequence of low energy intake or body pool mobilization23. However, palmitic acid concentration in milk fat seems to result from its content in the diet20, because if body fat had been mobilized, the milk oleic acid concentration should have increased, since this FA is the most concentrated in the body reserve[24]. In this work, the oleic acid content was similar in the milk of all three herd types. Nevertheless, this hypothesis cannot be ruled out since the oleic acid in milk can also be obtained from *de novo* synthesis via the desaturation of stearic acid in the mammary gland[25].

Although grass intake was not estimated for this study, a direct relationship between daily grazing time and grass intake has been observed[8]. Therefore, it can be assumed that cows in outdoor management eat more grass than cows in mixed management. However, according to data pro- vided by farmers in this study, the TMR intake was similar in indoor and mixed farms, which is in agreement with previous studies[26]. Therefore, elevated rumenic and vac- cenic acid contents in outdoor and mixed herd milk are associated with fresh forage consumption. This confirms the results of several authors who studied the effect of grazing-based management systems on milk FA concentration[27]; although it should be noted that some authors have not found differences in the rumenic acid concentration of milk produced by cows under indoor and outdoor systems[28].

The high proportion of unsaturated FAs, especially lin- olenic acid, in fresh forage would explain the increase of linolenic acid in the milk of cows feeding on grass, despite being the PUFA most susceptible to ruminal biohydrogena- tion[29]. Up to 99% of dietary linolenic acid intake may be totally or partially hydrogenated in the rumen, synthesizing vaccenic and stearic acids[30]. Vaccenic acid is desaturated in the mammary gland producing rumenic acid with the addition of a double bond between carbon 9 and 10[31]. The CLA : C18:1 *trans*-11 ratio serves as a marker of D-9 desaturase activity[32]. When fresh forage was included in the diet, this index was higher, reflecting greater desaturase activity in grazing cows. The finding of increased vaccenic and rumenic acids in the milk of the mixed and outdoor herds is consistent with that described by other authors[7]. This shows that fresh forage intake can improve the con- centration of healthy FAs in milk, while feeding on high proportions of concentrates and conserved forage reduces their content.

The lower concentration of SFAs and the higher concentration of UFAs in the outdoor and mixed herd milk agree with the results of other studies in which fresh forage was included in the diet[7,8]. The SFA : UFA ratio of the indoor herd milk (2.09) was above the current dietary limit of 2.0[33], while in the outdoor and mixed herd milk it was less than 2. The *n*-3 FA concentration increased signifi- cantly in the milk of the mixed and outdoor herds, while the *n*-6 FA concentration was not affected by the management system. However, the *n*-6 : *n*-3 ratio in the mixed and outdoor herd milk was 6.7, while in the indoor herd milk possessed a ratio of 11.8, which exceeded FAO dietary recommendations regarding essential FA intake[33].

The differences in milk fat and protein concentrations between the indoor and mixed/outdoor systems were in agreement with Bargo et al.[18]. However, our results con- trast with other authors, who reported lower fat and protein contents in the milk of herds fed preserved forage (maize silage, grass silage and cereal straw) and concentrates[27] or TMR[34] than in herds whose diets included fresh forage. Other authors report no differences in the fat and protein contents of the milk of cows fed TMR or grass[19]. The TMR of the present indoor herds was based on preserved forage and a high level of concentrates. This could have increased the metabolizable energy (ME) intake as well as the con- centration of propionate in the rumen, and it would result in an increase in milk protein[35]. In this paper, the TMR offered in indoor farms had higher, although not significant, net energy concentration than TMR in mixed and outdoor farms. In general, the first limitation to milk protein synthesis in grazing systems is the amount of ME available since grass has an excess of degradable protein[36]. Thus, the differences in the protein concentration of the milk of the outdoor herds compared to that of the indoor herds might be due mainly to the formers' low ME and/or degradable protein intake. In this sense, increasing ME intake increases the rate of microbial protein synthesis and propionate rela- tive to acetate in the rumen[20], resulting in increased rates of synthesis of milk protein, lactose, and to a lesser degree, fat in the mammary gland[35]. This may have occurred with indoor management.

CONCLUSION

Fat and protein in milk increased under the indoor feeding system, but milk FA profiles improved for both the mixed and outdoor systems. Therefore, milk from herds whose diets included fresh forage from grazing was of better quality in terms of total FA content, SFA and SFA : UFA ratio. Grazing appears to increase linolenic, vaccenic and rumenic acids in milk, while maintaining the *n*-6 : *n*-3 ratio within current nutrition recommendations.

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TABLES

Table 1. Differences in production variables (means – standard error) and proportion of feed used in each management system. Data are based on farm records and survey responses from a questionnaire.

Herd characteristics	Indoor	Mixed	Outdoor
Number of farms	10	4	6
Herd size (milking cows)	57 – 10.8	37 – 17.0	65 – 13.9
Milk yield per cow (kg lactation^{-1})	9715 – 351	8504 – 544	8631 – 453
TMR intake (kg fresh matter d^{-1})	45.6 – 2.46	47.5 – 6.95	31.6 – 3.48
Proportion of farms using the indicated feed			
Fresh forage	0.00	1.00	1.00
Maize silage	0.90	0.25	0.50
Grass silage	1.00	0.25	0.83
Grass hay	0.50	0.00	0.00
Alfalfa	0.40	0.00	0.00
Straw[1]	0.30	0.00	0.17
Cereals[2]	1.00	0.75	0.83
By-products[3]	0.00	0.50	0.00

[1] Wheat, barley and/or oat.
[2] Mixed concentrate feeds.
[3] Sugar beet pulp or apple pulp.

Table 2.Chemical composition (% dry matter basis) and FA profiles of the feed and pasture under different herd management systems

	TMR					Pasture			
	Indoor	Mixed	Outdoor	SEM[1]	*P*	Mixed	Outdoor	SEM[1]	*P*
Dry matter (DM)	47.7	48.7	49.8	1.26	NS	15.0	15.3	0.96	NS
Organic matter	90.5	90.3	91.3	0.17	NS	89.7	90.8	0.32	NS
Crude protein	16.4^{a}	14.2^{b}	12.7^{c}	0.23	***	20.0	20.7	1.72	NS
Neutral detergent fiber	43.4	46.9	46.4	0.58	NS	44.3	39.3	1.08	NS
Acid detergent fiber	23.0^{b}	25.1^{ab}	27.5^{a}	0.36	***	22.2	20.4	0.76	NS
Crude fat	4.46^{a}	3.72^{b}	3.69^{b}	0.088	**	2.70	3.54	0.306	NS
Starch	16.5	16.7	17.1	0.44	NS	ND	-	-	-
Net energy (Mcal kg^{-1} DM)	1.24	1.19	1.21	0.006	NS	1.65	1.74	0.029	NS
Fatty acids (g 100 g^{-1} FA)									
C6:0	0.10	0.24	0.22	0.029	NS	0.36	0.25	0.063	NS
C8:0	0.20^{b}	0.68^{a}	0.19^{b}	0.040	*	0.09	0.03	0.015	NS
C10:0	0.05^{b}	0.21^{a}	0.07^{b}	0.003	***	ND	ND	–	–
C12:0	0.33	0.47	0.30	0.026	NS	0.12	0.13	0.036	NS
C14:0	0.85^{b}	1.53^{a}	1.00^{b}	0.045	**	0.38	0.38	0.041	NS
C15:0	0.05	0.06	0.07	0.003	NS	ND	ND	–	–
C16:0	35.98	34.85	29.00	1.283	NS	23.16	26.30	1.510	NS
C16:1	0.20^{b}	0.19^{b}	0.30^{a}	0.006	**	0.16	0.36	0.133	NS
C17:0	0.09	0.07	0.07	0.005	NS	0.09	0.2	0.018	NS
C17:1	0.001	0.007	ND	0.001	NS	ND	ND	–	–
C18:0	2.97^{a}	2.85^{a}	1.31^{b}	0.184	*	1.55	0.88	0.224	NS
C18:1 *cis*9	27.34^{a}	27.33^{a}	18.84^{b}	0.562	**	2.77	2.76	0.363	NS
C18:2 *cis*9 *cis*12	27.59^{b}	26.17^{b}	42.98^{a}	1.776	*	16.86	15.70	1.585	NS
C18:3 *cis*9 *cis*12 *cis*15	4.13	2.85	5.58	0.370	NS	45.24	52.08	3.700	NS
C20:0	0.13	0.12	0.08	0.013	NS	0.37	0.22	0.031	NS
C20:2	0.002	ND	ND	–	–	ND	ND	–	–
C22:0	0.001^{b}	0.013^{a}	0.000^{b}	0.001	**	0.23	ND	–	–

[a,b,c]Means in a row with unlike letters differ significantly. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS: not significant.

[1] SEM, standard error of the means.

[2] ND, not detected.

Table 3. Milk composition and FA profile according to herd management system.

	Indoor	Mixed	Outdoor	SEM[1]	*P*
Protein (%)	3.14^{a}	3.06^{b}	3.07^{b}	0.008	***
Fat (%)	3.57^{a}	3.46^{b}	3.45^{b}	0.015	**
Lactose (%)	4.76^{a}	4.71^{b}	4.68^{b}	0.009	**
Urea (mg dl^{-1})	29.4^{a}	26.9^{b}	25.6^{b}	0.509	**
Fatty acids (g100 g^{-1} FA)					
C6:0	3.01	2.97	2.96	0.017	NS
C8:0	1.65	1.62	1.58	0.012	NS
C10:0	3.21^{a}	3.09^{b}	3.01^{b}	0.034	*
C11:0	0.12	0.11	0.10	0.004	NS
C12:0	3.47^{a}	3.51^{a}	3.20^{b}	0.037	**
C13:0	0.049	0.047	0.038	0.002	NS
C14:0	10.70^{a}	10.41^{ab}	10.11^{b}	0.075	**
C14:1	0.85	0.81	0.78	0.015	NS
C15:0	0.95^{b}	1.04^{a}	0.95^{b}	0.013	*
C16:0	30.16^{a}	27.58^{c}	28.74^{b}	0.214	***
C16:1	1.82^{a}	1.69^{b}	1.68^{b}	0.020	**
C17:0	0.80^{b}	0.94^{a}	0.90^{a}	0.021	*
C17:1	0.12^{b}	0.17^{a}	0.14^{ab}	0.005	**
C18:0	13.36^{b}	13.93^{a}	13.84^{a}	0.084	**
C18:1 *cis*9	24.44	25.24	25.07	0.150	NS
C18:1 *trans*11	1.81^{b}	2.86^{a}	2.71^{a}	0.074	***
C18:2 *cis*9 *cis*12	2.59	2.45	2.68	0.048	NS
C18:2 *cis*9 *trans*11	0.47^{b}	0.97^{a}	0.86^{a}	0.033	***
C18:2 *trans*9 *trans*12	0.07	0.08	0.09	0.003	NS
C18:3 *cis*9 *cis*12 *cis*15	0.25^{b}	0.41^{a}	0.42^{a}	0.013	***
C18:3 *n*-6	0.013	0.011	0.011	0.001	NS
C20:0	0.05	0.06	0.05	0.002	NS
C20:2	0.010	0.005	0.000	0.002	NS
C20:4	0.08	0.05	0.09	0.007	NS
SFAs	67.56^{a}	65.32^{b}	65.51^{b}	0.208	***
UFAs	32.43^{b}	34.67^{a}	34.49^{a}	0.208	***
n-6 : *n*-3 ratio	11.84^{a}	6.29^{b}	7.17^{b}	0.504	***

[a,b,c]Means in a row with unlike letters differ significantly.

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$. NS, not significant.

[1] SEM, standard error of the means.