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Effect of feeding flax meal on milk fatty acids profiles and performance of Holstein dairy cows

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ABSTRACT

This study evaluated the effect of flax meal (FM) and barley rootlets (BR) in Holstein dairy cows compound feeds (CFs) in order to assess their effects on the quality (physico-chemical parameters and fatty acids composition) and quantity (yield) of raw milk. The trial used a number of 15 multiparous Holstein dairy cows, at 27 weeks mid-lactation stage, averaging 624.19 kg of BW, with an initial average milk yield of 22.36 litres/day. The cows were divided in 3 groups (C, FM and FMBR diet, respectively), for 63 days. Cows were fed twice/day, and received a basic concentrate mix (corn, soybean meal, sunflower meal and wheat bran) and roughage (alfalfa hay and corn silage). The use of FM in dairy cows' CFs resulted significant increase of milk fatty acids (FA) and milk yield. At the end of the trial for both experimental groups (FM and FMBR), the C18: 3n3 FA was significantly higher, (0.526% and 0.522% vs. 0.23% C group of total FAMEs), representing a good source of n3. This indicates that the fatty acid addition from FM was favourable to the C18: 3n3 fatty acid in milk.

Keywords: Barley rootlets, Flax meal, Milk, Milky cows, n6/n3, Polyunsaturated fatty acid

Food consumption resulting from feeding animals with feeds containing FM (*Linum usitatissimum*) as a source of polyunsaturated fatty acids (PUFAs), including milk, has been associated with positive effects on the lipid profile of human blood (Malpuech-Brugère *et al.* 2010). FM contains 39.29% to 68.57% of C18: 3n3 FA (Quezada and Cherian 2012, Vlaicu *et al.* 2018). PUFA n3 FA, represented by C18: 3n3, showed some cardio-protective effects, prevents platelet aggregation, blood clots and cholesterol and also have both antithrombotic and anti-inflammatory effects and were associated with low incidences of cardiovascular diseases such as coronary artery disease and stroke (Leeson and Caston 1996, Nash *et al.* 1995, Simopoulos, 1996). Since milk and dairy products are a substantial part of the human fat source, it would be beneficial to increase the n3 FA content (Gantner *et al.* 2015). FM as a source of n3 FA has been widely used as it is well known for its abundant C18: 3n3 content (Scholljegerdes *et al.* 2014). It has also been shown that the intake of flax seed or oil by ruminants reduces methane production (Chilliard *et al.* 2009) and improves reproductive performances (Santos *et al.* 2008, Zachut *et al.* 2010b). The addition of FM in dairy cows' diets, changes the composition of milk FA by lowering the ratio of SFA and increasing the ratio of MUFA and PUFA representing an attractive source for inclusion in rations of dairy cows as a source of energy and protein (Petit 2003). Like other oilseeds, high levels of FAs in FM may adversely affect ruminal fiber digestion (Palmquist and Jenkins 1980). Regarding the barley rootlets (BR), they play an important role in cows feeding. The level of CP from BR (17.77%) is better than that from corn and lower than wheat, but it is nevertheless considered to have the lowest quality and digestibility (Zardo and Lima 1999). Cardova *et al.* (2004), in a study on dairy cows, tested the replacement of maize with barley by-products and found that the average proportion of barley inclusions increased NDF and ADF. For these reasons, several trials on dairy cows have been conducted to study the effect of using different flax forms (seed, meal and oil) to compare their effects with other sources on milk production and composition. In this context, the purpose of the study was to improve the concentration ratio of n3 FA from milk by replacing the sunflower meal (SM) with the FM and to improve the milk yield by partially replacing the wheat bran (WB) with BR in dairy CFs.

MATERIALS AND METHODS

Experimental procedures were approved by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition, in accordance with Romanian Law no. 305/2006 regarding handling and protection of animals used for experimental purposes. The experiment was carried out in accordance with the Romanian legislation (Law 206/2004, ordinance 28 / 31.08.2011, law 43 / 11.04.2014, Directive 2010/63 / EU).

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Table 1. Diets and compound feeds formulation and supply of nutrients

Item	Groups		
	C	FM	FMBR
Diet formulation (kg/cow/day)			
Alfalfa hay	6.00	6.00	6.00
Corn silage	30.00	30.00	30.00
CFs	8.00	8.00	8.00
Compound feed formulation (%)			
Corn	25.00	25.00	25.00
Bran	13.00	13.00	13.00
Soybean meal	12.00	12.00	12.00
Sunflower meal	12.00	0.00	0.00
Flax meal	0.00	12.00	12.00
Wheat bran	34.60	34.60	17.30
Barley rootlets	0.00	0.00	17.30
Calcium	1.10	1.10	1.10
Salt	1.30	1.30	1.30
Vitamin-mineral premix	1.00	1.00	1.00

Animals, experimental design and dietary treatments: Fifteen lactating Holsteins cows, with different parities, at 27 weeks mid-lactation stage, were used in a replicated (n = 5) 3 × 3 Latin square experiment with 63 days period, including 14 days of adaptation and last 7 days of data collection. Cows were housed in tie-stalls, fed twice/day keeping the diets isoenergetic and isonitrogenous and had free access to water. Cows were milked twice daily at 05:30 and 17:30 h, and the initial daily milk yield was 22.36 litres/day (expressed as average). The animals averaging 624.19 kg of BW were assigned randomly to 3 homogenous groups of 5 cows per group and received the same basal diet (6 kg alfalfa hay and 30 kg corn silage). Dietary treatment consisting of three CFs differentiated by the fat and protein ingredient. Control (C) group received a CFs consisting of corn, soybean meal, wheat bran, mineral premix and sunflower meal (12% in the CFs). In addition to the C diet, FM group which received the same basal diet and compound feed, 100% of the SM was replaced by FM (12% in the CFs); FMBR group, received also the same basal diet and CFs, in which the SM was replaced completely by FM (12% in the CFs) but also the WB from C diet was replaced 50% with BR (17.3% in the CFs). Diets (basal plus CFs addition) were formulated according to the nutritional requirements adapted to live weight, milk production, fat percentage and physiological status (Burlacu *et al.* 2002) (Table 1). All three new diets had the same nutritional characteristics: 19.07 DM (kg/cow/day), 18.33 milk feed units (FUmilk/cow/day), 1808.29 intestinally digestible protein by the nitrogen content (IDPN g/cow/day) and 1782.13 intestinally digestible protein by the energy content (IDPE g/cow/day).

Sample collection, analysis and Statistics

Compound feeds compositions and raw materials: One experimental batch/group was manufactured; CFs, raw materials and milk samples were collected and assayed for the basic chemical composition of the main nutrients. Standardized methods (according to CE Regulation 152/2009) were used to determine nutrients as follows: dry matter (DM), by the gravimetric method, drying at 103°C, using Sartorius scales and BMT drying oven, ECOCELL Blueline Comfort; crude protein (CP), by Kjeldahl method using the semiautomatic KJELTEC auto 2300 system – Tecator (Sweden); ether extractives (EE) by extraction in organic solvents, with SOXTEC-2055 FOSS system – Tecator (Sweden); crude fibre (CF) by the method with intermediary filtration, using FIBERTEC 2010 system – Tecator; ash (Ash) by the gravimetric method, using Caloris CL 1206 furnace.

Milk sampling: The milk samples were collected during the last week of the experimental period, for seven days in a row, both at the morning and evening milking (7×2 samples which means 14 samples per cow resulting 35 samples per diet). The milk from the same cow of morning and evening milk were mixed, the average value was used and were send for FA composition analyses.

Fatty acids: The FAs were determined by gas chromatography (SR CEN ISO/TS17764-2: 2008). The method involves the transformation of the FAs from the sample of fat into methyl esters, followed by the separation of the components in a capillary chromatograph column and their identification by comparison with standards chromatograms. We used a Perkin Elmer-Clarus500 chromatograph with capillary column injection and high polarity stationary phase (BPX70, 60 m × 0.25 mm inner diameter, 0.25 µm film), or high polarity cyanopril phases, which give similar resolution for different geometric isomers (THERMO TR-Frame 120 m × 0.25 mm ID × 0.25 µm film).

Statistical analysis: The analytical data were compared using variance analysis (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The experimental results were expressed as mean values with standard errors of means (SEM). The fatty acids were expressed as percentage of the total fatty acids as methyl ester. The differences being considered statistically significant for $P<0.05$

RESULTS AND DISCUSSION

Nutrient composition of the CFs and raw materials: The primary chemical composition of raw materials and CFs is presented in Table 2. The three CFs had similar amounts of nutrients, being balanced with iso-energy and iso-nitrogenous. The basic chemical analysis of raw materials revealed a variable CP level in FM, SM and BR. The highest CP level was determined in FM (39.35%) while the BR had only 15.53%. But by comparing the two oleaginous raw materials (FM and SM) and the two protein raw materials (BR and WB), the values were close. SM had the highest fat content (16.28%) being with 24.50% higher compared with the fat from FM (12.29%), while the BR had the lowest fat (1.45%) content, being with 91.03% lower than SM and with 88.20% lower than FM. Crude fiber was 32.40% for SM, 8.33% FM, 16.16% BR and 8.12% WB.

Table 2. Milk yield and chemical composition

Item	Diet			SEM	P value
	C	FM	FMBR		
Milk yield (litres/day)					
Initial	22.38	22.56	22.13	0.430	0.532
Final	23.48[a]	24.81[b]	25.02[b]	2.054	0.492
Chemical composition of milk (g/100 g DM)					
DM	14.10	12.87	12.59	1.314	0.156
OM	58.38	59.49	56.87	5.975	0.809
CP	3.14[a]	2.95[ab]	2.84[b]	0.234	0.965
EE	4.74	3.76	3.64	0.943	0.172
Ash	0.65	0.66	0.63	0.070	0.814

Values bearing different superscripts within the same row are different. DM, dry matter; OM, organic matter; CP, crude protein; EE, crude fat.

The obtained values are in agreement with the litreature data, although the raw materials have a lower level of chemical composition stability. Panaite *et al.* (2016) found that the FM had 20.91% crude protein and 7.18% crude fiber. The SM had 16.60% CP, 42.10% EE, 26.60% NDF and 21.30% ADF (Petit 2003). The chemical composition of the analysed raw materials allowed them to be integrated into the CFs for animal feeding, resulting in increased feed quality by intake of vitamins, minerals, fatty acids and other nutrients. The FA profile from raw materials, published previously (Vlaicu *et al.* 2017) showed that the FM is particularly rich in PUFA n3 (70.33 g/100 g FA) and n6 MUFA (19.51 g/100 g FA), Also, the data shows that the FM have a high level of linolenic acid C18: 3n3 (68.57%), as also shown by n6/n3 ratio (0.15), which makes FM represents an attractive concentrate for inclusion in rations of dairy cows as a source of energy and protein (Petit 2003).

Effect of fat sources on milk yield and composition: Milk-yield and physico-chemical composition of milk are shown in Table 2. A significant (P<0.05) effect of treatments was observed on final milk yield. The FM and FMBR groups had a significantly (P<0.05) higher milk yield quantity compared with C group. These results suggest that the FM had similar effects on both experimental groups, but also the specific essential amino acids have not been limited by the new CFs. It is already well known that FM when is included in dairy cows CFs, it can improve milk yield (De Vries 2006). Among milk physico-chemical parameters, no differences (P>0.05) were observed for DM, OM, or EE. Protein concentration in milk (Table 2) of experimental groups was significantly lower (P=0.965), which agrees with the decrease observed by Kennelly and Khorasani (1992). Protein concentration is usually lower for cows fed with supplemented fats, such as FM or SM. According to Vasilachi *et al.* (2018) fat is the most variable parameter of milk, not the protein, because it may be easily influenced by the animal nutrition.

Effect of fat sources on milk fatty acids composition: By using FM in dairy cows CFs had a significant (P<0.05) effect on milk FA content as presented in Table 3. The effect

Table 3. Milk fat fatty acids composition

Item	Diet			SEM	P value
	C	FM	FMBR		
% of total FAMEs					
C 4: 0	0.066[a]	0.043[b]	0.050[b]	0.020	0.250
C 6: 0	1.215	1.207	1.220	0.164	0.985
C 8: 0	1.351	1.550	1.529	0.162	0.062
C 9: 0	0.037	0.040	0.057	0.018	0.190
C 10: 0	3.296	3.759	3.874	0.409	0.014
C 11: 0	0.348	0.407	0.419	0.063	0.196
C 12: 0	3.969	4.356	4.700	0.507	0.025
C 13: 0	0.130	0.143	0.150	0.037	0.477
C 14: 0	13.070	13.790	13.940	1.013	0.121
C 14: 1	1.372	1.495	1.500	0.243	0.425
C 15: 0	0.529	0.579	0.580	0.092	0.382
C 15: 1	1.279	1.404	1.461	0.240	0.227
C 16: 0	36.280	34.240	36.510	3.247	0.231
C 16: 1	2.424	2.394	2.244	0.316	0.407
C17: 0	0.464	0.469	0.489	0.073	0.731
C 17: 1	0.574	0.646	0.587	0.087	0.140
C 18: 0	8.952	8.230	7.710	1.411	0.141
C 18: 1n9c	19.67	20.170	18.160	2.380	0.144
C18: 2n6t	0.371	0.562	0.580	0.149	0.007
C18: 2n6c	2.357	2.078	1.956	0.435	0.104
C18: 3n6	0.065	0.085	0.069	0.031	0.326
C 18: 3n3	0.234[a]	0.526[b]	0.522[b]	0.164	<0001
C 20: 0	0.068	0.061	0.068	0.025	0.799
CLA (C9, t11)	0.319	0.324	0.292	0.048	0.288
C20: 2n6	0.096	0.076	0.088	0.017	0.030
C20: 3n6	0.074	0.107	0.082	0.030	0.388
C20: 3n3	0.079	0.089	0.087	0.019	0.471
C20: 4n6	0.141	0.128	0.112	0.030	0.974
Others	1.175	1.036	1.022	0.161	0.058
Milk fatty acids profile (% of total FAMEs)					
SFA	69.770	68.880	71.27	3.053	0.214
MUFA	25.320	26.100	23.95	2.545	0.161
UFA	29.050	30.080	27.70	2.964	0.203
PUFA, of which:	3.727	3.974	3.755	0.588	0.605
Ω3	0.314[a]	0.613[b]	0.611[b]	0.171	<0.001
Ω6	3.095	3.037	2.850	0.494	0.526
Ω6/Ω3	10.010[a]	4.983[b]	4.746[b]	0.463	<0.001
SFA/UFA	2.448	2.314	2.595	0.356	0.214
PUFA/MUFA	0.148	0.152	0.155	0.018	0.693

Values bearing different superscripts within the same row are different.

was almost similar in both diets where FM and FMBR was used, compared with C diet. Even if the diets included a high proportion of UFA, ruminant milk contain much higher levels of SFA due to extensive bio-hydrogenation of dietary UFA in the rumen (Shingfield *et al.* 2010). Also, fatty acids *de novo* synthesis accounts for all butyric (C4: 0) to lauric (C12: 0), most of the myristic (C14: 0; approximatively 90%) and about 50% of palmitic (C16: 0) acids are secreted in milk, whereas all C18 carbon and longer chain fatty acids are thought to be derived from circulating plasma lipids (Chilliard *et al.* 2000). Although, the new diets did not influence significantly the UFA proportion. The lower values of stearic (C18: 0) and C18: 1n9c acids from the milk fat of cows fed FMBR diet and C represents a positive aspect from nutritional point of view. The FMBR diet led to a lower levels of medium-chain MUFA, particularly of pentadecenoic (C15: 1) and heptadecenoic (C17: 1) acids compared with FM group, while palmitoleic (C16: 1) and C18: 1n9c had comparable values between all three groups. The C16: 0, considered by the World Health Organization in the same group with the trans acids responsible for the higher risk of cardiovascular diseases (Voicu *et al.* 2017), decreased with 5.62% in the milk fat of cows fed FM diet compared to C and FMBR diet. This fact should be taken into consideration especially because the C16: 0 together with C12: 0 and C14: 0 are known as reputed atherogenic acids. The FM diet and FMBR diet compared to C diet decreased the content of cis-6 linoleic acid (C18: 2n6c) with 11.83% and 17.01% respectively compared with C. The diets containing FM, had a significant influence of α linolenic acid (C18: 3n3), in the milk concentration. The FM diet increased slightly the milk content of total CLA, by 1.20 times while the FMBR decreased, and also the arachidic acid (C20: 0) tended to decrease in FM group. These results were similar with those obtained by Abu Ghazaleh *et al.* (2004). As for eicosadienoic (C20: 2n6) and eicosatrienoic acids (C20: 3n6 and C20: 3n3, respectively), no significant differences were observed between treatments, even if the C20: 3n6 and C20: 3n3 had higher values in FM and FMBR groups compared with C. The two diets containing FM as the main ingredient, are equivalent for use in dairy cows' feeds. Thereby, FM as a FA sources may enhance animals final products closer to human requests. Although significant amounts of PUFAs are given to dairy cows, the outflows into the milk fat are limited to about 10% or less. However, these results were satisfactory because the milk fat n6/n3 ratio was significantly ($P<0.05$) lower (by 50.51% and 52.58%) in the cows fed FM and FMBR diet. According to Kumar *et al.* (2016), the long-chain PUFAs play many roles in human nutrition. PUFAs, based on the position of the first double link, are of two types: n3 and n6 fatty acids. From the PUFAs, the long-chain linolenic acid (18: 2) is a major n6 fatty acid while 18: 3 (α-linolenic acid), is a major n3 fatty acid.

Feeding dairy cows with flax meal beneficially altered the milk fatty acid composition, as indicated by higher concentrations of health-promoting CLA and C18: 3n3. Results of this study showed that most of the effects observed for dairy cow performance, were related to the milk yield. The milk yield was increased significantly by the use of flax meal and flax meal with barley rootlets. However, the lack of some effects of flax meal on milk fatty acids may be associated with the low inclusion rate and extensive ruminal biohydrogenation.

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