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Milk production, milk fatty acid composition, and blood biochemical parameters of Holstein dairy cows fed whole or ground flaxseed instead of extruded soybeans in the first half of lactation

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Summary

To evaluate the effects of physically processed flaxseed [ground flaxseed (GFS) and whole flaxseed (WFS)] as a replacement for extruded soybeans on feed intake, milk production, milk composition, milk fatty acid (FA) composition and blood biochemical parameters, nine primiparus Holstein dairy lactating cows averaging 495 ± 34.5 kg of body weight (BW) and 70 ± 5 days in milk (DIM) were assigned in a 3 × 3 change over Latin square design with three periods. Intake of dry matter was similar among the animals fed the treatments, also milk yield and milk composition were not influenced by the diets. Processing of flaxseed significantly ($P<0.05$) affected plasma glucose and urea concentrations. Feeding ground compared with whole flaxseed and extruded soybeans, caused a significant ($P<0.05$) increase in the concentration of total long-chain fatty acids in milk, also α-linolenic acid (18:3) content nearly doubled with GFS compared with those of the other treatments (0.75 vs. 0.36 and 0.46 g/kg milk production for GFS, ESB or WFS, respectively). Linoleic acid (18:2), content of milk was also significantly ($P<0.05$) increased by inclusion of the ground flaxseed compared with the other treatments. In general, feeding ground flaxseed had a significant ($P<0.05$) effect on monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) concentrations compared with those of the cows fed extruded soybeans or whole flaxseed.

Key words: Dairy cow, Flaxseed, Fatty acid, Milk production

Introduction

Dietary polyunsaturated fatty acids (PUFA) are perceived to be healthier than saturated fatty acids (SFA) (Petit *et al*., 2004). Flaxseed is known to increase the concentration of PUFA in milk, but usually does not exceed 3 to 4% of total fatty acids (Kennelly, 1996). Feeding flaxseed generally results in the lowest omega 6 to omega 3 FA ratio in milk fat (Petit, 2002), which may improve milk FA profile and result in better human health. In general, untreated whole flaxseed is readily eaten by dairy cows, and feeding up to 150 g/kg DM as flaxseed had no effect on DM intake of early (Petit, 2002) and late lactation (Martin *et al*., 2008) dairy cows. Previous experiments were carried out using either ground (Gonthier *et al*., 2005) or whole flaxseed (Petit *et al*., 2002). Physical breakdown of flaxseed in dairy cow rations could increase milk production (Kennelly, 1996). Indeed, in a short-term experiment (i.e., 3 week periods), dairy cows fed 100 g/kg DM ground flaxseed had a 6.5% increase (1.2 kg/d) in milk production compared with those fed 100 g/kg DM whole flaxseed (da Silva *et al*., 2007). The objective of this experiment was to determine the effects of substitution of extruded soybeans with whole and ground flaxseed on performance, milk FA profile and blood parameters of lactating Holstein dairy cows.

Materials and Methods

Experimental design and data collection

In a 3 × 3 change over block design a total of 9 primiparus Holstein dairy cows averaging 495 ± 34.5 kg of body weight (BW) and 70 ± 5 days in milk (DIM) were randomly assigned to one of the three experimental rations (Table 1). Rations (TMR) contained different oil seed sources including extruded soybeans (ESB), ground flaxseed (GFS) and whole flaxseed (WFS) were provided. Cows were housed in tie stalls and fed individually. Each experimental period consisted of 24 days of adaptation to the diets and 7 days for daily data collection. Between each experimental period, a wash out of 15 days was performed, in which cows consumed the no linseed diet and treatments were performed when there was no significant difference among cows for measurements. Three cows were located in each block (B=3) and cows within each block consumed a similar ration in each period. Cows were milked three times daily at 5:30, 13 and 20:30 h. Milk production was recorded at days 24 to 29 of each experimental period. Body weight (BW) and body condition score (BCS) of each cow were determined at the end of each experimental period.

Table 1: Ingredient and chemical composition of total mixed experimental diets of Holstein lactating dairy cows fed extruded soybeans (ESB), whole flaxseed (WFS) or ground flaxseed (GFS)

Ingredient composition (g/kg DM)	Treatments		
	ESB	WFS	GFS
Corn silage	142	142	142
Alfalfa hay	280	280	280
Barley grain (ground)	140	140	140
Corn grain (ground)	124	124	124
Extruded soybeans	106	-	-
Whole flaxseed	-	97	-
Ground flaxseed	-	-	97
Wheat bran	77	85	85
Rape seed meal	62	62	62
Soybean meal	54	55	55
Sodium bicarbonate	5	5	5
Mineral and vitamin supplement	10	10	10
Chemical composition (g/kg)			
Crude protein	179	178	178
Ether extract	44	45	45
Neutral detergent fiber	330	331	331
ME (MJ/kg DM)	11.6	11.5	11.5

Rations were formulated to meet or exceed the requirements of a 500 kg cow at 70 DIM, producing 37 kg/d of milk with 3.5% fat. Dry matter, protein, fat and fatty acid composition of the oilseeds used in the current study are presented in Table 2. Diets were fed individually twice daily at 7:00 and 18:00 h at *ad libitum* rates to allow 100 g/kg refusals. Each feed ingredient and samples of TMR were collected, frozen and pooled for each collection period (days 24 to 31). The pooled samples were then mixed thoroughly and sub samples were provided for further chemical analyses (Rezaii *et al*., 2010, 2011). The nitrogen content of feed ingredients as well as TMRs was determined using Kjeldahl method (AOAC, 1990) in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Fatty acid profiles of the oilseeds were determined as described by Sukhija and Palmquist (1988).

Table 2: Chemical composition of oil seed sources (DM basis)

Item	Oil seeds	
	Flaxseed	Soybeans
CP, % of DM	27.2	39.1
Ether extract, % of DM	31.4	24.4
Fatty acids, % of total fatty acids		
C16:0	7.45	11.9
C18:0	4.39	3.3
C18:1	18.21	20.8
C18:2	16.47	56.9
C18:3	52.23	7.1

Milk samples were obtained at days 26 and 27 of each experimental period. The samples were collected at three consecutive milkings, mixed in proportion to yield appropriate composted samples, then analysed to determine the milk chemical composition for fat, protein, lactose and solid not fat (SNF) using a milkoscan analyser (Foss Electric, Conveyor 4000). Concentration of fat, protein, lactose and SNF, and SCC in milk was determined using milkoscan analyser (Foss Electric, Conveyor 4000). Milk fat extraction and fatty acid separation were performed according to a procedure described by Chouinard *et al*. (1999). Fatty acid methyl esters were prepared by trans methylation, and were then quantified by using a gas chromatograph (GC system 6890, Hewlett-

Packard, Wilmington, DE) equipped with a flame-ionization detector and a CP-7489 fused-silica capillary column (100 m × 0.25 mm i.d. with 0.2-µm film thickness; Varian, Walnut Creek, CA). Initial oven temperature (50°C) was held for 1 min then ramped at 5°C/min to 160°C, where it was held for 42 min, and then ramped at 5°C/min to 190°C and held for 22 min. Inlet and detector temperatures were maintained at 250°C, and the split ratio was 100:1. Hydrogen carrier gas flow rate through the column was 1 ml/min. Hydrogen flow to the detector was 30 ml/min, airflow was 400 ml/min, and the nitrogen make-up gas flow was 25 ml/min. Peaks in the chromatogram were identified and quantified using pure methyl ester standards.

Blood samples were collected on day 31 of each experimental period before and 4 h after the morning feeding. Samples of blood were taken from the jugular vein in vacutainer tubes containing sodium heparin. Tubes were immediately placed on ice and then centrifuged at 4°C for 15 min at 3500 × g. These samples were used to determine plasma concentration of glucose, urea, non esterified fatty acids (NEFA), β-hydroxyl butyric acid (BHBA), glutamic oxaloacetic transeaminas (GOT) and Glutamic pyrovic transeaminase (GPT) using colorimetric methods (A15, Biosystems, S.A., Spain).

Statistical analysis

Data were analysed using GLM procedure of SAS (2000) as a 3 × 3 change over block design with the following model:

$$Yijkl = \mu + B_i + C_{j(i)} + P_k + T_l + e_{ijkl}$$

where,

Yijkl: The dependent variable

µ: Overall mean

B_i: Random effect of block (i=3)

$C_{j(i)}$: Random effect of cow within block (j=3)

P_k: Fixed effect of period (k=3)

T_l: Fixed effect of treatment (l=3)

e_{ijkl}: Random residual error

Significance was declared at $P<0.05$.

Results

Dry matter intake was not different between the treatments (Table 3). Treatments had no effect on BW and BCS (Table 3). Milk yield and milk composition were not influenced by the diets (Table 3). Plasma concentrations of NEFA, BHBA, GOT and GPT did not significantly differ between the diets in all sampling times (Table 4), but glucose concentration was significantly lower for animals fed GFS in both times of before and 4 h after the morning feeding compared with those fed WFS and ESB. Four h after morning feeding, blood urea concentration was significantly lower in the cows fed GFS and WFS compared with those fed ESB.

Dietary treatments had a significant impact on milk fatty acid composition (Table 5). Long-chain fatty acids in milk were lower in ESB and WFS compared with GFS (P<0.05). The results showed that α-linolenic acid (18:3) content of milk was nearly twice as high (P<0.05) in GFS compared with ESB and WFS (0.775 vs. 36.2 and 0.46 g/kg milk for GFS, ESB and WFS, respectively). Linoleic acid (18:2) content of milk had significantly higher

Table 3: Feed intake, milk production, milk composition, and feed efficiency of Holstein lactating dairy cows fed extruded soybeans (ESB), whole flaxseed (WFS) or ground flaxseed (GFS)

Item	Treatments			SEM*	P**
	ESB	WFS	GFS		
Dry matter intake (kg DM/d)	25.9	24.8	25.2	0.71	NS
Milk yield (kg/d)	30.5	30.9	30.6	0.76	NS
Feed efficiency (milk/feed intake)	1.2	1.2	1.2	0.04	NS
Milk fat (g/kg)	30.7	32.8	31.4	1.13	NS
Milk protein (g/kg)	28.8	28.0	30.2	0.89	NS
Milk lactose (g/kg)	45.2	46.2	45.8	0.60	NS
Milk SNF[1] (g/kg)	87.8	88.4	89.3	1.15	NS
Body weight (kg)	509	512	510	12.0	NS
Body condition score(BCS)	2.7	2.7	2.8	0.13	NS

[*] SEM: Standard error of mean, [**] P: Probability. [1]: Solid not fat

Table 4: Plasma blood biochemical parameter of lactating Holstein cows fed diets containing extruded soybeans (ESB), whole flaxseed (WFS) or ground flaxseed (GFS)

Item	Treatments			SEM*	P**
	ESB	WFS	GFS		
Before morning feeding					
Glucose (mg/dl)	64.67[a]	62.45[ab]	60.53[b]	1.463	<0.05
Urea (mg/dl)	41.54	42.29	41.78	3.326	NS
NEFA[1] (mmol/L)	0.162	0.183	0.171	0.030	NS
BHBA[2] (mmol/L)	0.221	0.279	0.236	0.036	NS
GOT[3] (U/L)	59.93	56.26	62.45	5.004	NS
GPT[4] (U/L)	30.71	31.98	33.65	2.570	NS
4 h after morning feeding					
Glucose (mg/dl)	66.16[a]	60.23[b]	58.80[b]	1.675	<0.05
Urea (mg/dl)	24.58[a]	18.38[b]	19.46[b]	3.605	<0.05
NEFA[1] (mmol/L)	0.087	0.100	0.112	0.015	NS
BHBA[2] (mmol/L)	0.476	0.435	0.485	0.050	NS
GOT[3] (U/L)	62.40	60.25	63.41	6.012	NS
GPT[4] (U/L)	33.06	33.28	32.89	2.410	NS

* SEM: Standard error of mean, ** P: Probability. a, b Row values with same superscript or no superscript are not significantly different (P<0.05). [1]: Non esterified fatty acid, [2]: β-hydroxyl butyric acid, [3]: GOT: Glutamic oxaloacetic transeaminas, and [4]: GPT: Glutamic pyrovic transeaminase

Table 5: Fatty acid-composition in the milk (g/kg milk production) of Holstein cows fed diets containing extruded soybeans (ESB), whole flaxseed (WFS) or ground flaxseed (GFS)

Item	Treatments			SEM*	P**
	ESB	WFS	GFS		
SCFA[1] (g/kg milk)	1.14	1.40	1.35	0.14	NS
MCFA[2] (g/kg milk)	11.14	12.18	11.73	0.66	NS
LCFA[3] (g/kg milk)	4.49[a]	4.69[a]	5.25[b]	0.27	<0.05
Monounsaturated FA[4] (g/kg milk)	9.89[a]	10.93[a]	11.89[b]	0.49	<0.05
Polyunsaturated FA[5] (g/kg milk)	2.10[a]	2.14[a]	2.78[b]	0.12	<0.05
C18:2 (g/kg milk)	1.84[a]	1.84[a]	2.402[b]	0.09	<0.05
C18:3n-3 (g/kg milk)	0.36[a]	0.46[b]	0.75[c]	0.03	<0.05

* SEM: Standard error of mean, ** P: Probability. a, b Row values with same superscript or no superscript are not significantly different (P<0.05). [1]: Short-chain FA: C4:0 to C10:0, [2]: Medium-chain FA: C11:0 to C17:0, [3]: Long-chain FA: >C18:0, [4]: C10:1, C14:1, C16:1, C17:1, C18:1 trans, C18:1cis, C20:1, C22:1, C24:1, and [5]: C18:2 trans, C18:2 cis, C18:3n-3

GFS than in ESB and WFS. Feeding ground flaxseed caused a significant (P<0.05) effect on monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) concentrations compared to the cows fed extruded soybeans or whole flaxseed.

Discussion

The present study showed that cows fed whole or ground flaxseed up to 9% of their diet DM had similar DMI compared with those which were fed extruded soybean. Previous studies showed that cows fed ground (Gonthier *et al.*, 2005) or whole (Petit *et al.*, 2004) flaxseed had similar DMI compared with those fed no flaxseed. Flaxseed supplementation had no effect on milk production. This is in agreement with Kennelly and Khorasani (1992) who used whole flaxseed at 0.0, 5, 10, or 15% of DMI without affecting milk yield. Similar milk production has been reported for cows fed 10.4-10.8% of DM whole flaxseed and those fed 17.7-18.4% of DM basis micronized soybeans for the first 16 weeks of lactation (Petit, 2002). This is in agreement with Kennelly and Khorasani (1992) who used whole flaxseed at 0.0, 5, 10, or 15% of DMI without affecting milk yield. Petit (2003) found that feeding cows either with whole untreated or whole formaldehyde-treated flaxseed or sunflower seed, had no effect on milk yield among the diets. Grinding of flaxseed enhanced milk production when it was added to the diet of dairy cows up to 10% of DM (da Silva *et al*., 2007). Similarly, rolling flaxseed increased milk production of early lactation cows fed 10% of DM basis flaxseed (Kennelly, 1996). In contrast, feeding inclusion of ground flaxseed at levels lower than 10% of ration had no significant effect on milk yield of mid lactation cows fed a high forage diet (Collomb *et al*., 2004). These results suggest that there is no beneficial effect of flaxseed processing on milk production when flaxseed is fed at low concentration, although physical breakdown of the seed may be required for enhanced milk production when flaxseed is supplemented at higher concentration (Petit and Cortes, 2010). Milk fat content did not differ between the dietary groups in the present study. Similarly, inclusion of whole flaxseed in the diet of dairy cows at levels ranging from 5 to 15% (Ambrose *et al*., 2006) and 10.8 to 12.0% (Petit and Benchaar, 2007) of the ration DM had no effect on milk fat proportion. Milk protein and lactose concentrations did not differ between the dietary groups in our study, which was in agreement with the findings of Petit and Cortes (2010). In addition, Oba *et al*. (2009) fed 10% of DM basis flaxseed in the diet as ground, rolled or whole seed and reported a non-significant difference when milk composition was considered.

No differences in plasma urea concentration were observed when ground flaxseed was compared with whole flaxseed or extruded soybeans in the samples taken before the morning feeding, but significantly decreased ($P<0.05$) in GFS and WFS. Physical breakdown of seeds is known to increase ruminal CP degradability (Stern *et al*., 1994). Therefore, greater ruminal CP degradability for ground flaxseed rather than the whole seeds may contribute to increased urea concentration in blood and milk, but the result of our study indicated lower plasma urea concentration by grinding, that might show better utilization of nitrogen by this group of animals (Mojtahedi and Danesh Mesgaran, 2011). There was a difference between diets in plasma glucose concentration at both times of sampling (before and 4 h after the morning feeding), in which the cows fed GFS diet showed a decrease compared with that of the other treatments. Conversely, da Silva *et al*. (2007) and Petit and Cortes (2010) reported similar plasma concentration of glucose by flaxseed grinding. The reason for decrease in glucose concentration in the cows fed GFS diet in our study was unknown, but because of the similarity in DMI, BCS and milk yield between all animals fed the experimental diets, the decrease in plasma glucose concentration in cows fed ground flaxseed might not have had a negative effect on energy balance of the cows. Present dietary treatments do not affect plasma concentrations of NEFA, BHBA, GOT and GPT. Blood concentration of NEFA is an index of body fat mobilization (Roberts *et al*., 1981), and is related to the energy balance of the dairy cows. Bertics *et al*. (1992) reported that DMI is inversely related to concentrations of NEFA and BHBA in plasma and liver triglyceride. Thus, the lack of a significant difference in NEFA and BHBA concentrations from the current study may be related to the similar DMI in the cows fed all treatments.

In the current study, an increase in long-chain fatty acid concentrations of milk was observed when cows were fed with GFS diet. Results confirmed the data reported by da Silva *et al*. (2007). Physical breakdown of flaxseed may contribute to an increase of availability of FA for absorption and transfer into milk, partly as a result of a higher ruminal passage rate of ground flaxseed compared with whole seed (da Silva *et al*., 2007). There was an increase of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) concentrations in cows fed GFS compared with those fed with ESB and WFS diets. Substantial increase in milk MUFA and PUFA in our experiment imply that sufficient quantities of PUFA of flaxseed escaped from bio-hydrogenation in the rumen and ultimately were transferred to the milk fat by physical breakdown of the seed. Petit and Cortes (2010) showed that grinding the flaxseed had no effects on concentration of milk PUFA; however, both whole and ground flaxseed increased PUFA content of milk compared with the no flaxseed diet. In our study, increase occurred in the proportion of both α-linolenic and linoleic acids in the cows fed GFS diet; most notable being α-linolenic acid, which nearly doubled in milk of cows fed ground flaxseed. According to Glasser *et al*. (2008), the proportion of 18:3 in milk fat increased by 79% with flaxseed supplementation compared with un-supplemented diets. Chilliard *et al*. (2009) reported that feeding 148 g/kg extruded flaxseed, compared with 124 g/kg whole flaxseed increased the proportion of cis 9, 12, 15-18:3 in milk fat, most likely because rupture of the seed coat increased the rate of oil released from the seeds into rumen fluid compared with intact seeds. Similarly, Glasser *et al*. (2008) reported that ground flaxseed feed increased the proportion of c18:3 FA in milk fat more than did the whole seeds. However, in some experiments, feeding cows with ground (Petit and Cortes, 2010) or rolled (Oba *et al*., 2009) flaxseed had no advantage over the whole seed in increasing c18:3 FA proportion of milk.

In conclusion, whole or ground flaxseed used in up to 9% of diet DM had no effect on DMI, BW and BSC of the Holstein lactating dairy cows. In addition, grinding of flaxseed did not affect milk yield and milk composition of Holstein dairy cows compared with those fed whole flaxseed or extruded soybeans. Similar plasma concentration of NEFA and BHBA between all treatments may show similarity in energy balances in all experimental units. Increased concentration of MUFA and PUFA, and most notably being α-linolenic acid, which nearly doubled in milk of cows fed ground flaxseed treatments, which would improve beneficial milk FA profile from a human health perspective.

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