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Milk production and composition, milk fatty acid profile, and blood composition of dairy cows fed different proportions of whole flaxseed in the first half of lactation

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ABSTRACT

A total of 32 Holstein cows were allotted at calving to eight groups of four cows blocked to determine the effects of feeding increased levels of whole flaxseed (WF) in the diet on dry matter (DM) intake (DMI), milk production and composition, milk fatty acid (FA) profile, concentration of some blood metabolites and energy balance. Cows within each block were assigned to one of four iso-net energy for lactation total mixed rations: no whole flaxseed (0WF), and diets with preplanned inclusions of 50 (50WF), 100 (100WF) or 150 (150WF) g/kg DM WF. Diets were fed for *ad libitum* intake from calving to week 24 of lactation. There was a trend for an interaction between treatment and week for DMI, milk yield, and logsomatic cell count (P=0.08, 0.07, and 0.09, respectively). Values of DMI averaged for the 24 week experiment, yields of fat, protein and total solids and proportions of short- and medium-chain FA in milk fat decreased linearly with higher proportions of WF in the diet. Milk yield was similar among diets. Proportions of 18:0, *cis*9-18:1, *trans*9-18:1, *cis*9,*trans*11-18:2, *cis*9,12,15-18:3 19:0 and 20:0 in milk fat increased linearly and those of *cis*9,12-18:2, *trans*9,12-18:2 and 20:4 decreased linearly with higher concentrations of WF in the diet. Although milk FA profile was enhanced, feeding more than preplanned inclusions of 50 g/kg DM WF had negative effects on yield of milk components.

1. Introduction

Flaxseed (*Linum usitatissimum*) supplementation has been shown to decrease embryo mortality in dairy cows (Petit and Twagiramungu, 2006). Feeding whole flaxseed (WF) during the transition period also contributes to decrease the incidence of the fatty liver syndrome as suggested by higher and lower concentrations of glycogen and triglycerides, respectively, in the liver of multiparous cows after calving (Petit et al., 2007). Indeed, flaxseed is likely a glucogenic energy source (Van Knegsel et al., 2007), thus it has the potential to reduce the risk for metabolic disorders in dairy cows. Most experiments on flaxseed have been short-term trials with less than 2 months or studies with processed flaxseed (*e.g.*, extruded, rolled, ground) and the optimum level of WF to incorporate in the dietary dry matter (DM) of cows from calving through the first half of lactation

Abbreviations: ADF, acid detergent fiber; DM, dry matter; DMI, dry matter intake; FA, fatty acid; aNDF, neutral detergent fiber; NEFA, non-esterified fatty acids; TMR, total mixed ration; TTAD, total tract digestibility; WF, whole flaxseed; 0WF, diet with 0 g/kg DM whole flaxseed; 50WF, diet with 50 g/kg DM whole flaxseed; 100WF, diet with 100 g/kg DM whole flaxseed; 150WF, diet with 150 g/kg DM whole flaxseed.

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is unknown. Thus, there is a need to provide more information on feeding WF for longer periods. Flaxseed is a good source of fat (314 g/kg DM; Petit, 2002). Rabiee et al. (2012) have recently reported that the final meta-regression model for DM intake (DMI) indicated that feeding fat for a longer period increased DMI, thus emphasizing the need for further long term experiments on fat (*e.g.*, flaxseed) supplementation. Therefore, the objective of this experiment was to determine the effects of feeding different proportions of WF on performance (*i.e.*, feed intake, milk production and milk composition) of dairy cows through the first half of lactation (24 weeks) and blood parameters related to the fatty liver syndrome during early lactation. Milk fatty acid (FA) profile also was examined. The hypothesis was that increasing levels of WF in the diet has no effect on performance of dairy cows.

2. Materials and methods

2.1. Cows, experimental design, and diets

The experiment was carried out at the Atlantic Dairy and Forage Institute (ADFI) of Fredericton Junction, NB, Canada, from May 2011 to February 2012 using 28 multiparous and 4 primiparous Holstein cows. The experiment was conducted from calving to week 24 of lactation (first half of lactation). Cows were blocked (4 cows per group) by parity and for calving dates expected within a 4 week period. There were 7 blocks of multiparous cows and one block of primiparous cows. Cows within groups were assigned randomly to one of four total mixed rations (TMR; Table 1) that consisted of a control TMR with no WF (0WF), and TMR with preplanned inclusions of 50 (50WF), 100 (100WF) or 150 (150WF) g/kg DM WF. All diets were designed to have similar concentrations of crude protein and net energy for lactation. Cows were housed in tie stalls and fed individually. Diets were fed twice daily at 07:00 and 15:00 h at *ad libitum* rates to allow 100 g/kg refusals and consumption was recorded daily. Cows were milked twice daily at 05:45 and 16:45 h and production was recorded at every milking. Cows were cared for in accordance with guidelines of the Canadian Council on Animal Care (CCAC, 1993). The body weight of each cow was determined weekly for the first 8 weeks of lactation and every 4 weeks afterwards.

Total collection of feces was carried out on week 10 of lactation from the first 8 blocks of cows to calve for determination of total tract apparent digestibility (TTAD). Milk yield and DMI are less variable after the lactation peak, which was the rationale behind the choice of week 10 of lactation for TTAD measurement. Feces were collected from a rubber mat placed behind the

Table 1
Ingredient and actual chemical composition of total mixed diets of Holstein cows fed no flaxseed (0WH), 50 g/kg dry matter (DM) whole flaxseed (50WF), 100 g/kg DM whole flaxseed (100WF) or 150 g/kg DM whole flaxseed (150WF).[a]

	Treatment			
	0WF	50WF	100WF	150WF
Ingredient composition (g/kg DM)				
Corn silage	273	265	265	265
Haylage[b]	265	285	285	274
Barley, grain (rolled)	89	89	88	87
Corn, grain (ground)	190	141	105	80
Soybean, meal (480 g/kg crude protein, solvent)	148	138	129	120
Flaxseed, whole	0	46	93	139
Megalac[c]	5	7	7	6
Limestone	6	5	5	5
Mineral and vitamin supplement[d]	24	24	23	24
Chemical composition (g/kg DM)				
Crude protein	17.4	17.6	17.4	17.6
Neutral-detergent fiber, ash free	35.9	36.1	35.9	35.8
Acid-detergent fiber, ash free	21.4	22.7	22.0	23.0
Net energy for lactation (MJ/kg DM)[e]	6.90	6.90	6.90	6.90
Fatty acid (g/kg DM)[f]				
14:0	0.1	0.1	0.1	0.2
16:0	5.6	7.3	8.4	9.7
18:0	0.7	1.4	1.9	2.5
*cis*9 18:1	5.1	8.9	11.9	14.6
*cis*6 18:2	10.0	12.5	13.5	15.1
*cis*3 18:3	4.4	13.3	21.6	27.9
Total fatty acids	26.4	44.5	59.0	71.2

[a] Mean of 10 monthly samples prepared by compositing weekly samples.
[b] Timothy grass.
[c] Church and Dwight Co. Inc., Princeton, NJ, USA.
[d] Contained (as fed basis) 143 g/kg of Ca, 23 g/kg of P, 65 g/kg of Mg, 15 g/kg of S, 55 g/kg of Na, 7 g/kg of K, 984 mg/kg Fe, 1772 mg/kg Zn, 496 mg/kg Cu, 1784 mg Mn, 33 mg/kg I, 53 mg/kg Co, 14 mg/kg Se, 256.990 IU/kg of vitamin A, 77,100 UI/kg of vitamin D_3, and 1697 IU/kg of vitamin E.
[e] Calculated using published values of feed ingredients (NRC, 2001).
[f] Mean of pool sample from eight samples prepared by compositing pool samples from seven daily samples collected from eight cows per diet during week 10 of lactation.

animals and stored in plastic containers. Daily feces were weighed and mixed thoroughly. A representative sample (20 g/kg) was taken and stored at −20 °C for subsequent freeze drying. Total daily urine was collected in stainless steel containers *via* Gooch tube (BF Goodrich Co., Kitchener, ON, Canada) attached to the cow with a nylon netting covered with neoprene (Spall Bowan Ltd., Guelph, ON, Canada) affixed to the vulva to avoid contamination with feces.

2.2. Sample collection and analyses

Samples of TMR were collected weekly, frozen, and composited on a 4 week basis. Composited samples were mixed thoroughly and subsampled for chemical analyses. Silage DM was analyzed weekly for DM adjustment of the TMR. Milk samples were obtained once every four weeks from each cow for two consecutive milkings and analyzed separately to determine milk composition. Milk samples were stored at +4 °C with a preservative (bronopol-B2) until analyzed 2 days later for fat, crude protein, lactose and somatic cell counts. Milk samples were collected without preservative for two consecutive milkings from the first 6 blocks of cows to calve on week 8 of lactation. Milk samples were pooled within cow relative to production to obtain one composite milk sample per cow and frozen at −20 °C until analyzed for milk FA profile. Blood was collected from all cows on week 1, 2, 3, 4, 5 and 6 postpartum 3 h after the morning feeding to determine non-esterified FA, β-hydroxybutyrate and glucose concentrations as described previously (Petit and Côrtes, 2010).

Dry matter of TMR was determined by drying samples in a vacuum oven at 100 °C overnight according to method 934.01 of the AOAC (1990). Diets were dried at 55 °C for 48 h and ground to pass a 1 mm screen in a Wiley mill before chemical analyses. Methods used for the determinations of neutral detergent fiber (aNDF), acid detergent fiber, and N in the TMR, non-esterified FA, glucose and β-hydroxybutyrate in plasma, concentrations of N, fat, lactose and total solids and somatic cell counts in milk have been described previously (Petit and Côrtes, 2010). Extraction and methylation of FA in milk and diets, and separation of FA methyl esters were performed as detailed by Petit and Côrtes (2010).

2.3. Statistical analysis

All results were analyzed using the MIXED procedure of SAS (2000) as a randomized block design with block and treatment as the main sources of variation and cow as the experimental unit. Data on DMI and milk production were averaged for each week of lactation. Repeated measurements were analyzed with the following model: $yijk = \mu + \alpha i + \beta j + \tau k + (\alpha\tau)ik + eijk$, where $yijk$ is the dependent variable, μ is the population mean, αi is the treatment effect, βj is the fixed effect of block, τk is the effect of time (week of lactation or hour), $(\alpha\tau)ik$ is the interaction effects of treatment and week of lactation or hour, and $eijk$ is the residual error. Treatment effects were partitioned to provide polynomial (linear, quadratic and cubic) contrasts.

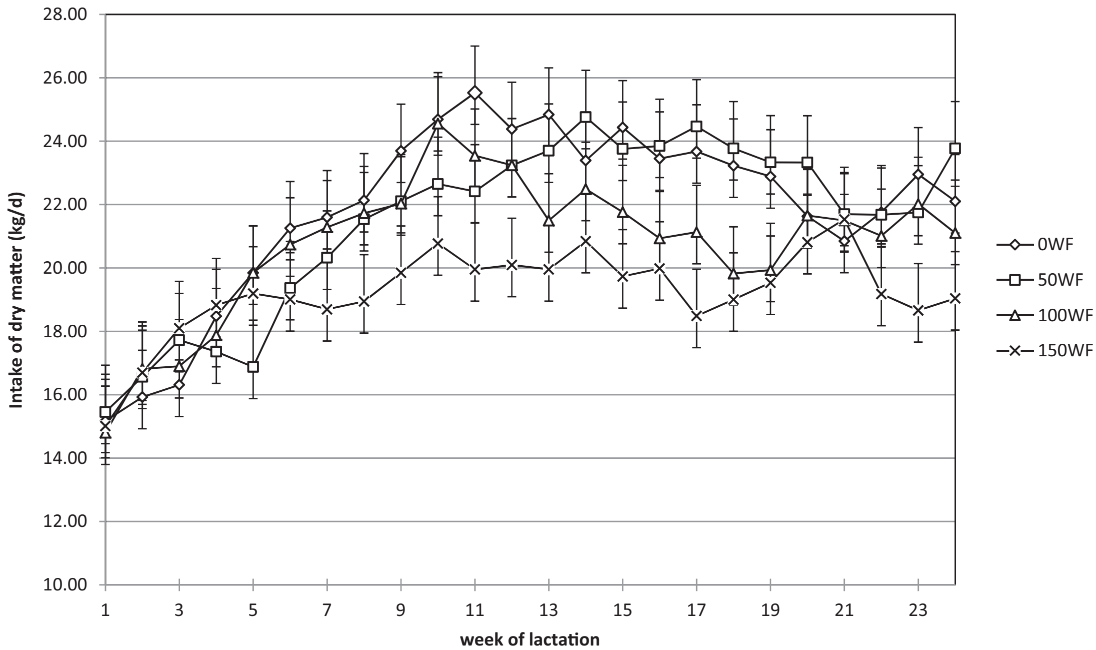

Fig. 1. Intake of dry matter (DM) of Holstein cows fed no flaxseed (◊), 50 g/kg DM whole flaxseed (□), 100 g/kg DM whole flaxseed (△) or 150 g/kg DM whole flaxseed (×).

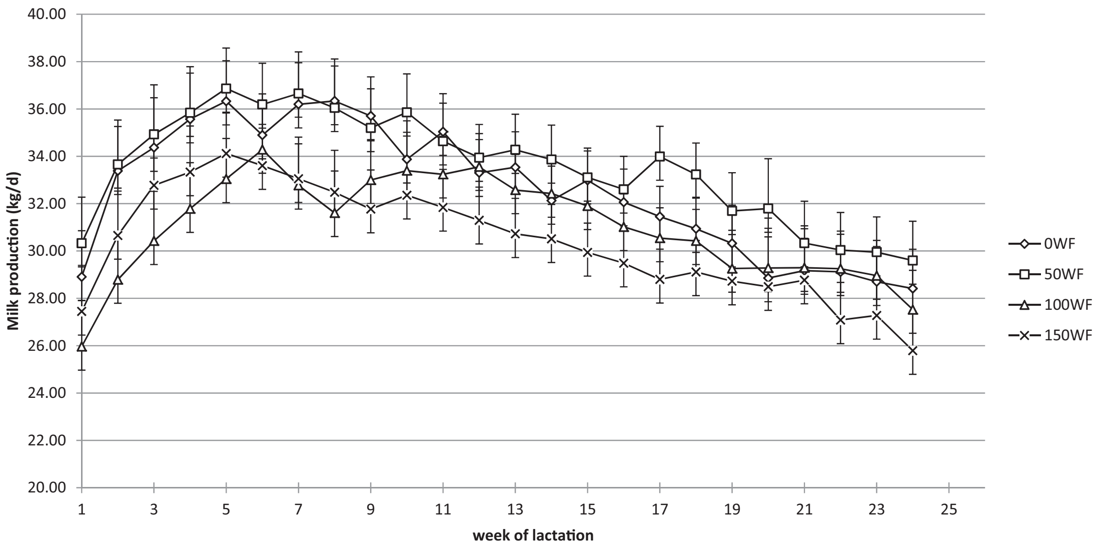

Fig. 2. Milk production of Holstein cows fed no flaxseed (◊s), 50 g/kg dry matter (DM) whole flaxseed (□), 100 g/kg DM whole flaxseed (△) or 150 g/kg DM whole flaxseed (×).

3. Results

The overall health status of cows during the first two weeks postpartum was good with only one cow with retained placenta (100WF), one with prolapse (100WF) and one with mastitis (CON). No other health problems were detected and no cows were treated for ketosis.

There was a trend (P=0.08) for an interaction between treatment and week for DMI, expressed in kg per d, as a result of a different evolution over time among treatments (Fig. 1). There was a linear effect of treatment on DMI (P=0.04), expressed in kg per d, and energy for lactation (P=0.03) averaged for the 24 week experiment as a result of a decrease with higher proportions of WF in the diet. When DMI was expressed in percentage of BW, there was no interaction between DMI and week of lactation and daily intake averaged for the whole experiment was similar among treatments. There was a trend (P=0.07) for an interaction between treatment and week for milk yield as a result of a different evolution over time among treatments (Fig. 2). However, yield of milk averaged for the first 24 weeks of lactation was similar (P=0.11) among treatments (Table 2). There was no interaction between treatment and week for proportions of fat and total solids. There was an interaction (P=0.02) between treatment and week for protein concentration as a result of linear decreases on week 16, 20, and 24 (P=0.04, 0.01, and 0.006, respectively) with higher proportions of WF in the diet (Fig. 3a). The interaction between treatment and week was significant (P=0.04) for lactose concentration as a result of linear increases on week 4, 8, 20, and 24 (P=0.04, 0.04, 0.10, 0.06, respectively) with higher proportions of WF in the diet (Fig. 3b). There was no interaction between treatment and week for yield of milk components. Milk yields of fat, protein and total solids decreased linearly with higher levels of WF in the diet. Proportions of fat and total solids and yield of lactose were similar among diets. The interaction between week and treatment tended (P=0.09) to be significant for logsomatic cell counts as a result of a different evolution over time among treatments. However, values of logsomatic cell counts averaged for the 24 week experiment were similar among diets.

There was no interaction between treatment and week for plasma concentration of β-hydroxybutyrate, glucose and non-esterified FA, and treatment had no effect (Table 2). The energy balance between week 4 and 24 of lactation was similar among treatments and values averaged 10.4, 8.2, 13.8, and 3.5 kJ/d for cows fed 0WF, 50WF, 100WF, and 150WF, respectively. There was no treatment effect on TTAD of DM, crude protein, ADF, and aNDF, and values averaged, respectively, 0.695, 0.699, 0.552, and 0.567.

Most milk FA proportions were affected by diets (Table 3). The only FA that were not different among diets were 4:0, *cis*9-16:1, 17:0, and 22:5. Proportions of short-(6:0, 8:0, 10:0, 11:0, and 12:0) and medium-chain FA (14:0, 14:1, 15:0, and 16:0) in milk fat were decreased linearly by feeding increased amounts of WF in the diet. Proportions of 18:0, *cis*9-18:1, *trans*9-18:1, *cis*9, *trans*11-18:2, *cis*9,12,15-18:3 19:0 and 20:0 in milk fat increased linearly and those of *cis*9,12-18:2, *trans*9,12-18:2 and 20:4 decreased linearly with higher concentrations of WF in the diet. Proportions of total *trans*, monounsaturated, polyunsaturated and n-3 FA increased linearly by feeding increased levels of WF while the inverse was observed for proportions of saturated and n-6 FA.

Table 2
Intake, body weight, milk production, milk composition and blood composition of Holstein cows fed no flaxseed (0WH), 50 g/kg dry matter (DM) whole flaxseed (50WF), 100 g/kg DM whole flaxseed (100WF) or 150 g/kg DM whole flaxseed (150WF).

	Treatment				SEM	P	
	0WF	50WF	100WF	150WF		Linear	Quadratic
Dry matter intake (kg/d)[a]	21.8	21.5	20.8	19.2	0.91	0.04	0.53
Dry matter intake (g/kg of body weight)[a]	3.62	3.31	3.63	3.27	0.167	0.33	0.87
Intake of net energy for lactation (MJ/d)	141.5	144.3	131.6	131.8	4.25	0.03	0.15
Initial body weight (kg)[a]	594	605	550	576	26.8	0.22	0.69
Final body weight (kg)	589	644	572	597	21.1	0.47	0.29
Body weight change (kg/24 wk)	−5	38	23	21	12.7	0.27	0.08
Milk production (kg/d)[a]	32.6	33.5	31.0	30.4	1.21	0.11	0.52
Milk composition (g/kg)[a]							
Crude protein	3.18	3.07	3.02	3.05	0.060	0.09	0.28
Fat	4.06	3.92	3.92	3.94	0.102	0.40	0.43
Lactose	4.57	4.57	4.61	4.68	0.041	0.06	0.40
Total solids	11.81	11.56	11.55	11.67	0.134	0.42	0.18
Milk yield (kg/d)[a]							
Crude protein	0.65	0.60	0.54	0.56	0.027	0.01	0.23
Fat	0.76	0.74	0.67	0.68	0.031	0.03	0.70
Lactose	0.95	0.90	0.84	0.87	0.044	0.15	0.33
Total solids	2.36	2.24	2.05	2.11	0.098	0.04	0.38
SCS[a,b]	4.73	5.05	4.84	4.82	0.204	0.95	0.40
Plasma concentration							
Non-esterified fatty acids (Eq/L)	0.26	0.26	0.28	0.26	0.023	0.91	0.74
Glucose (mmol/L)	3.19	3.19	3.21	3.20	0.080	0.89	0.98
β-Hydroxybutyrate (mol/L)	680	715	644	629	60.0	0.41	0.67

[a] Mean for the first 24 weeks of lactation.
[b] Somatic cell score = log 10 somatic cell count.

Table 3
Fatty acid profile in milk (g/kg total fatty acids) of Holstein cows fed no flaxseed (0WH), 50 g/kg dry matter (DM) whole flaxseed (50WF), 100 g/kg DM whole flaxseed (100WF) or 150 g/kg DM whole flaxseed (150WF).

	Treatment				SEM	P	
	0WF	50WF	100WF	150WF		Linear	Quadratic
4:0	2.4	2.2	1.8	2.6	0.22	0.88	0.03
6:0	7.7	7.7	6.4	6.3	0.29	0.0003	0.86
8:0	11.1	10.1	8.0	7.8	0.50	<0.0001	0.29
10:0	26.0	24.7	17.8	17.7	0.92	<0.0001	0.35
11:0	2.6	2.4	2.0	1.8	0.13	0.0002	0.94
12:0	30.9	28.5	20.1	20.0	1.11	<0.0001	0.21
14:0	117.6	112.5	91.5	84.4	3.30	<0.0001	0.98
14:1	7.5	6.9	6.3	5.5	0.49	0.005	0.91
15:0	10.7	8.8	8.1	8.3	0.63	0.005	0.07
16:0	345.7	314.4	280.6	246.4	6.56	<0.0001	0.74
*cis*9-16:1	3.4	3.4	3.0	3.1	0.17	0.11	0.61
17:0	4.2	3.7	4.6	4.7	0.73	0.50	0.61
18:0	128.8	154.9	174.8	187.6	7.34	<0.0001	0.50
*cis*9-18:1	237.1	253.8	293.8	313.1	8.93	<0.0001	0.58
*trans*9-18:1	24.7	26.4	36.3	43.4	2.44	<0.0001	0.15
*cis*9,12-18:2	21.1	19.2	19.3	17.6	0.98	0.02	0.70
19:0	1.0	1.6	3.5	4.3	0.43	<0.0001	0.80
*trans*9,12-18:2	2.3	1.7	1.3	1.2	0.15	<0.0001	0.06
20:0	1.7	2.0	2.8	2.5	0.15	<0.0001	0.04
*cis*9,12,15-18:3	4.1	5.3	7.6	8.9	0.53	<0.0001	0.76
*cis*9,*trans*11-18:2	4.5	5.0	5.7	7.5	0.53	<0.0001	0.05
20:4	1.3	1.1	0.8	0.9	0.09	<0.0001	0.08
22:5	0.7	0.8	0.7	0.8	0.08	0.36	0.40
Total *trans*	31.5	33.1	42.7	47.9	2.51	<0.0001	0.35
Others	2.9	2.9	3.2	3.6	0.13	<0.0001	0.42
MUFA[a]	265.7	279.6	310.8	336.8	9.86	<0.0001	0.51
PUFA[a]	39.8	41.3	48.5	55.3	1.94	<0.0001	0.18
SFA[a]	699.1	682.0	614.3	600.5	11.22	<0.0001	0.97
n-3[b]	5.3	6.8	8.7	10.4	0.53	<0.0001	0.76
n-6[c]	23.0	20.6	19.7	18.6	1.00	0.005	0.52

[a] MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[b] *cis*9,12,15-18:3+*cis*11,14,17-20:3+*cis*5,8,11,14,17-20:5+22:5+22:6.
[c] *cis*9,12-18:2+*cis*6,9,12-18:3+*cis*8,11,14-20:3+*cis*5,8,11,14-20:4.

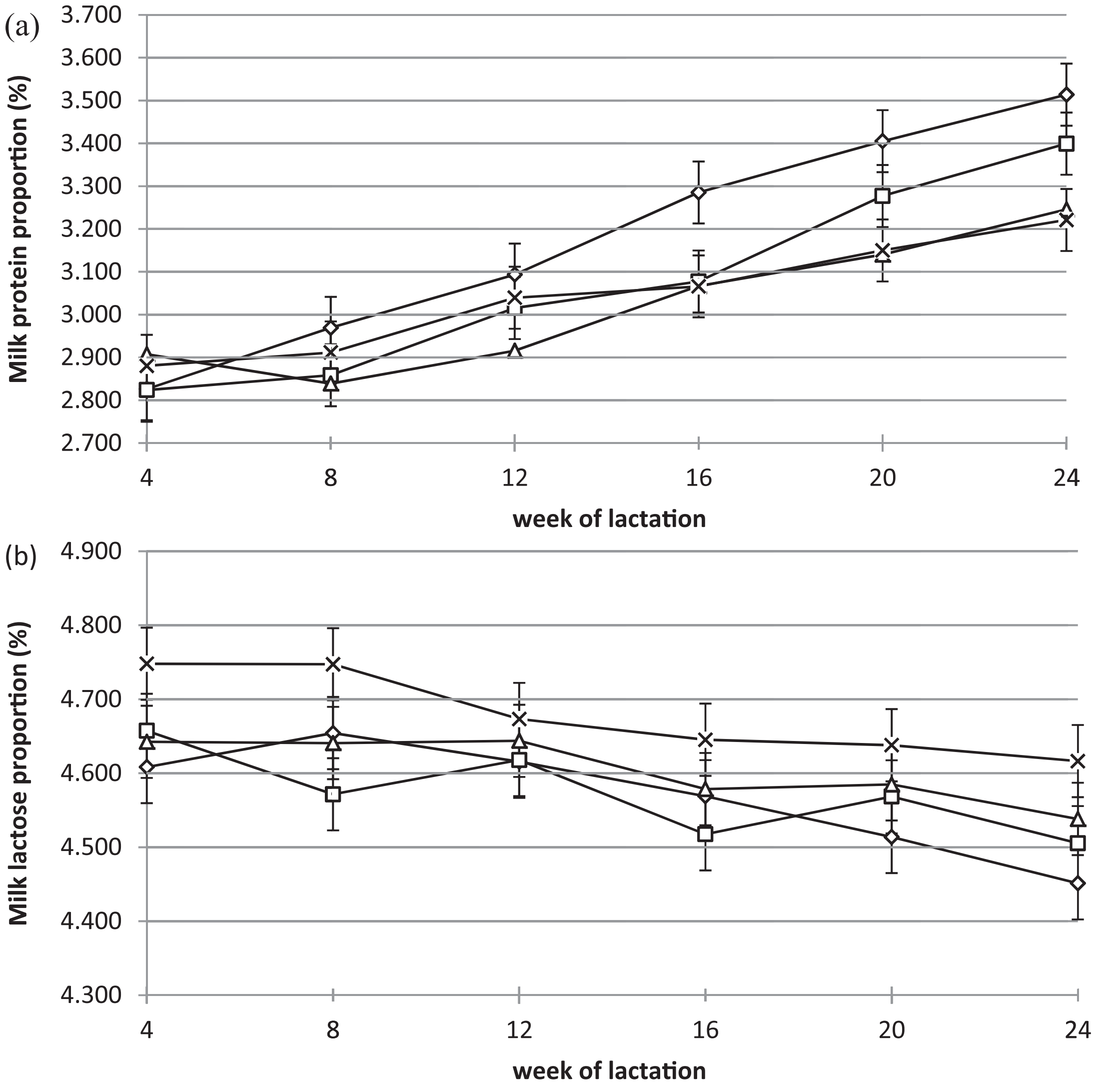

Fig. 3. Proportion of protein (a) and lactose (b) in milk of Holstein cows fed no flaxseed (◊), 50 g/kg DM whole flaxseed (□), 100 g/kg DM whole flaxseed (△) or 150 g/kg DM whole flaxseed (×).

4. Discussion

The similar mean DMI, expressed as a percentage of BW, and milk yield among diets agree with the results of Petit and Gagnon (2009) who fed 142 g/kg WF and those of Chilliard et al. (2009) who added 124 g/kg DM in a 4 × 4 Latin square design with 4 week periods. According to Martin et al. (2008), feeding WF has no negative effect on DMI and milk yield due to a slow release of FA in the rumen fluid as compared to when flaxseed is fed extruded or as free oil.

The lack of effect of increasing dietary proportions of WF on digestibility agrees with the results of Schroeder et al. (2014). A recent study (Côrtes et al., In Press) revealed that the amount of WF recovered from the feces of dairy cows fed 50, 75 or 100 g/kg DM WF increased in parallel with the dietary concentration of flaxseed, thus explaining that TTAD of nutrients from flaxseed recovered from the feces remained similar. Conversely, cows fed a corn silage-based diet (>500 g/kg DM) supplemented with 124 g/kg DM WF had lower TTAD of DM and organic matter than those fed no flaxseed (Martin et al., 2008). These authors speculated that the negative effects of lipids on digestion is more pronounced with corn silage diets than with hay diets likely due to the high availability of rapidly degradable carbohydrates in corn silage, which can reduce ruminal pH and decrease rate of fiber digestion.

The lower milk protein concentration from week 16 of lactation with higher WF levels in the diet disagrees with the similar protein concentration and yield in milk reported for mid-lactating cows fed diets containing 50–150 g/kg DM WF (Petit and Gagnon, 2009; Petit et al., 2009). Differences in stage of lactation and milk production are likely factors which moderate the response of cows to added dietary fat as a result of energy not being limited for dairy cows in the mid stage of lactation and those with low milk yield. Moreover, it has been shown that feeding diets of 125–127 g/kg DM flaxseed reduces microbial crude protein flow to the duodenum and microbial efficiencies (true and apparent) in dairy cows later on in lactation, thus decreasing the amount of microbial protein supply for milk protein synthesis (Gonthier et al., 2004).

The increase in lactose concentration with higher dietary proportions of WF on week 4, 8, 20, and 24 of lactation agrees with the higher lactose concentration obtained for dairy cows fed 72 g/kg DM flaxseed (either whole, ground or a mixture of both) compared to those fed no flaxseed (Petit and Côrtes, 2010). Earlier results have shown that linolenic acid, which represents more than 550 g/kg of total FA in flaxseed (Petit, 2002), results in one of the highest rates of gluconeogenesis among long chain FA (Mashek and Grummer, 2003). As ruminants depend on gluconeogenesis for lactose production (Bell and Bauman, 1997), higher gluconeogenesis with WF supplementation may contribute to increase lactose concentration in milk. Indeed, recent results (Mach et al., 2013) have shown that extruded flaxseed supplementation at 130 g/kg DM changes the expression of glucose transporter 2 in the liver of cows without changing DMI, suggesting that more glucose was secreted and probably available for lactose synthesis with a direct effect on energy metabolism of dairy cows.

Yields of fat decreased with higher proportions of WF in the diet as a result of similar percentages of fat among diets and numerical decreases ($P=0.11$) in milk yield with increased amounts of WF in the diet. The lack of WF supplementation on milk fat proportion agrees with similar milk fat concentrations reported previously for early lactation cows fed from 97 to 114 g/kg DM WF (Petit et al., 2004; Petit and Benchaar, 2007). The similar energy balance observed among diets agrees with the lack of treatment effects on plasma concentrations of β-hydroxybutyrate, glucose and non-esterified FA and the results of Petit and Côrtes (2010) who reported no effect of feeding 72 g/kg DM WF for the first 6 weeks of lactation on plasma concentrations of β-hydroxybutyrate.

Changes in milk FA proportions agree with the meta-analysis of the response of cow milk FA composition to oilseed lipid supplements demonstrating that dietary inclusion of flaxseed reduces the concentration of short-chain (C4 to C12) FA and C16:0 while the concentration of monounsaturated and long-chain FA increases (Glasser et al., 2008).

5. Conclusions

Yield of milk averaged for the first 24 weeks of lactation was similar among diets but evolution over time was different. Yields of fat, protein, and total solids decreased with higher levels of WF in the diet. Feeding increased proportions of WF in the diet increased proportions of total *trans*, monounsaturated, polyunsaturated and omega-3 FA in milk fat. Levels of WF in the diet had no effect on TTAD of nutrients, plasma concentrations of β-hydroxybutyrate, glucose, and non-esterified FA, and energy balance. Although milk FA profile was enhanced, feeding more than 46 g/kg DM WF had negative effects on yield of milk components. Therefore, evaluation must be carried out to decide which factor between FA profile or yield of milk components is the most profitable from an economic point of view.

Conflict of interest

None declared.

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