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Improving Milk Nutritional and Environmental Value with Flaxseed-Supplemented Diets

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Methane (CH_4) is an important greenhouse gas which is responsible for 20% of global warming [1]. 40% of methane originates from agriculture, and enteric methane from ruminant livestock enteric emission contributes 3–8% to the total greenhouse gas effect [2, 3]. Reducing methane emissions from polygastric animals presents significant opportunities for improving the environment as well as cattle feed efficiency since methane emissions represent a loss of energy for animals [4, 5].

Enteric methane output is linked to acetate (C2) production in the rumen, and for milking cows C2 is the main precursor used for saturated fatty acids (SFA) production in the cow's udder. Therefore, by decreasing C2 production in the rumen, there will be a resulting dual benefit of simultaneously decreasing methane output and SFA yields from dairy cows [2]. More precisely, microbial anaerobic digestion in the rumen produces hydrogen, and methanogenesis is the main pathway for removing this hydrogen generated during C2 and β-hydroxybutyrate (C4) synthesis in the rumen [6]. Two volatile fatty acids (FA), C2 and to a lesser extent C4, are used in the de novo FA synthesis pathway leading to C4:0-C16:0 production in the mammary epithelial cells [7].

While a decrease in enteric methane production is important for the environment, it is also linked to farm economics as methane output is a waste of energy for dairy cows [5]. Methane output per liter of milk is inversely and strongly correlated to milk yield [8]. Consequently, researchers have tried several strategies to reduce methane output with different additives [9].

As described above, methane production is at the crossroads between methanogenesis and lipogenesis. Numerous studies having shown positive results decreasing methane output with C18:3n–3-enriched diets through flaxseed supplementations [10, 11]. High dietary C18:3n–3 decreases C2 and C4 production in the rumen reducing hydrogen production and inhibiting methane synthesis through a toxic effect of C18:3n–3 itself (more precisely of its hydrogenated by-products resulting from ruminal fermentation) on bacteria and protozoa involved at the different steps of methanogenesis [11, 12].

Some studies have reported a biological and mathematical correlation between methane output and milk FA composition [12, 13]. This relationship was confirmed in in vivo trials where a strong correlation between milk FA composition for SFA and methane output was reported

[12, 13]. Therefore, enriching cows diets with C18:3n–3 from flaxseed holds great promise because (1) C18:3n–3 is the main FA in grass and has a long tradition in cows' diets [14–16], (2) C18:3n–3 sources like grass or flax are low input crops, and (3) C18:3n–3 is known to reduce C2 and C4 production in the rumen [17–19]. It is also well-established that C18:3n–3 deficiencies in human diets can be linked to health and reproduction/fertility problems [20]. As dairy lipids are the most abundant source of lipids in human western diets, even a modest enrichment of C18:3n–3 in milk can have positive consequences on human nutrition and health [21, 22].

The aim of this paper is to demonstrate that supplementing the diets of dairy cows with flaxseed improves the nutritional quality of the milk and reduces methane output.

Table 1. Composition of flax supplements

	Flax meal	Tradilin 70 NOP
Humidity	9.0	9.2
Crude protein	33.1	18.9
Total fat	13.6	28.4
Available fat	67.0	80.0
C18:2n–6	17.3	27.6
C18:3n–3	50.4	57.4

Humidity, crude protein and total fat are expressed as a percent of total matter. Available fat is expressed as a percent of total fat. C18:2n–6 and C18:3n–3 are expressed as a percent of total FA.

Table 2. Flax supplementation during the experimentation (from January 2009 to June 2009)

	Flax meal	Extruded
January	0.0	0.0
February	1.1	0.5
March	1.8	0.9
April	2.1	0.9
May	2.1	0.9
June	pasture	pasture

The weight of flax supplementation is expressed in kg/cow/day.

Methods

Experimental Design

Fifteen organic farms volunteered to participate in the trial which ran from January 2009 to May 2009. Organic production was chosen because of its low impact on several aspects relating to carbon footprinting. However, this does not necessarily mean a low contribution to global warming due to relatively high enteric methane output [23–26]. Farms were selected from organic dairy producers from the CROPP Cooperative who were suppliers for Stonyfield Farm and willing to participate in the trial. All of the farms were based in the Northeast USA in Vermont and New Hampshire. Stored grass (silage or hay) was the main forage on all these farms. Diets were very consistent between farms: a mix of grass silages with an adapted pellet or mash (supplied by Morison feed, Barnet, Vt., USA) to reach isoenergetic and isoproteic values in control and experimental periods. January 2009 was considered as the control month (control: diet without flax supplementation). Two experimental groups were then created: one with flax meal supplementation [flax meal diet (FM), 11 farms] and one with extruded flax supplementation [extruded flaxseed diet (EF), 4 farms]. The composition of FM and EF are shown in table 1. Flaxseed sources were introduced in the diets on the basis of isoconcentrate substitution from February 2009 to the end of the trial (table 2). In February 2009, the diets progressively reached the targeted level of 250 g of total dietary C18:3n–3 per cow and per day. To reach this level, flax was supplemented on winter diet forages. In the FM group, the targeted supplementation was 2.7 kg of NOP (National Organic Program) with flax meal. Flax intake of each individual farm depended on forage quality (particularly protein level). For the EF group, the targeted supplementation was 0.9 kg of an organic extruded mix: TradiLin® NOP with 70% flax and 30% wheat (Valorex, Combourtillé, France).

Milk Fatty Acid Profile

Gas chromatography was run monthly on tank milk for each individual farm by the Department of Agriculture of the University of Vermont from January 2009 to April 2009. An aliquot of milk was stored without preservatives at –20°C until analyzed for FA composition. Milk lipids were extracted using the method of Hara and Radin [27] and FA methyl esters prepared by base-catalyzed transmethylation [27, 28]. FA methyl esters were quantified using a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector and a flame-ionization detector using a CP-Sil 88 WCOT fused silica column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Varian Inc., Lake Forest, Calif., USA). Gas chromatographic conditions have been described by Kramer et al. [29].

Methane Output

Methane output was calculated from milk FA composition using three different equations described by Chilliard et al. [12] (based on milk SFA content) and Weill et al. [30] (based on milk FA composition and milk yield) as follows:

- Equation 1 [12]: CH_4 (g/day) = 16.8 × C16:0 (% of total FA) – 77, where C16:0 is expressed in percent of total FA.
- Equation 2 [12]: CH_4 (g/day) = $9.97 \times \Sigma FA_{(C8:0\ to\ C16:0)} - 80$, where $\Sigma FA_{(C8:0 to C16:0)} = C8:0+C10:0+C12:0+C14:0+C16:0$ (expressed in percent of total FA).
- Equation 3 [30]: CH_4 (g/l of milk) = $\Sigma FA_{(C4:0\ to\ C16:0)} \times 11.37 \times (MY)^{-0.04274}$, where $\Sigma FA_{(C4:0\ to\ C16:0)}$ = FA with 16 carbon atoms or less (expressed in percent of total FA) and MY = milk annual yield (kg/cow/year).

Statistical Analysis

Differences between treatments were examined with one-way ANOVA using Statistica 9.0 (Statsoft Inc., 1984–2009). The model included the treatment effect. Differences between means were compared using the Bonferroni's multiple comparison tests. Results are presented as least squares means for the treatment group together with the root mean square errors. The level of significance was set at $p < 0.05$.

Results

Milk Fatty Acid Composition

Effect of Flax

The proportion of SFA in milk decreased significantly in the flax diets compared with the control diet (–6%, $p < 0.001$; table 3). This global decrease by the introduction of flaxseed in the diet is visible in table 3. The decrease for short-chain FA like C4:0, C6:0, C8:0 and C10:0 [5% ($p < 0.01$), 5% ($p < 0.001$), 8% ($p < 0.05$) and 9% ($p < 0.001$), respectively] was lower than the decrease observed for medium SFA like C12:0, C14:0 and C16:0 [11% ($p < 0.01$), 9% ($p < 0.001$) and 13% ($p < 0.001$), respectively]. Finally, C18:0 increased significantly from 22% ($p < 0.001$).

The proportion of monounsaturated FA increased by about 9% ($p < 0.001$). This increase could be explained by the augmentation of cis-9 C18:1 (11%, $p < 0.001$) and trans-11 C18:1 (28%, $p < 0.01$). No significant difference was observed for trans-10 C18:1 ($p < 0.10$).

Although there is no significant difference ($p < 0.10$) concerning the proportion of polyunsaturated FA, we observed simultaneously a mild decrease in C18:2n–6 15%, $p > 0.01$) and an important increase in C18:3n–3 (31%; $p > 0.001$), resulting in a decrease in the C18:2n–6/C18:3n–3 ratio from 2.3 to 1.6.

Finally, the CLA (mainly cis-9 trans-11 C18:2; data not shown) also reaches a quite high level of 1.02% with a +22% ($p < 0,001$) increase.

The Effect of Processing

The introduction of EF instead of FM significantly decreased ($p > 0.01$) SFA from 64.4 to 62.6 for FM and EF, respectively (table 3). The proportion of monounsaturated FA ($p > 0.01$) increased significantly from 24.0% in the FM group to 25.7% in the EM group. Likewise, polyunsaturated FA ($p > 0.05$) increased from 2.8% in the FM group to 3.0% in the EF group.

The effect of the process on the FA proportion is associated with a diminution of the proportion in the milk of C14:0 (FM 10.4%, EF 9.9%; $p < 0.05$), a tendency to reduce the proportion of C16:0 (FM 28.8%, EF 27.3%; $p < 0.10$), a tendency to increase the proportion of trans-11 C18:1 (FM 20.6%, EF 22.1%; $p < 0.10$), and an increase of C18:2n–6 (FM 1.4%, EF 1.6%; $p < 0.05$) and C18:3n–3 (FM 0.9%, EF 1.0%; $p < 0.05$).

Methane Output

The introduction of flaxseed to the diets of dairy cows significantly decreases the production of methane. Using the equation of Chilliard et al.

Table 3. Effects of flaxseed supplementation in experimental diet of dairy cows on milk FA composition

	Control diet	Flax diet		Statistics			Percent change	
		flax meal	extruded flax	RMSE	flax effect	feed form effect of flax	C-FM	C-EF
C4:0	2.1	2.0	2.0	0.1	**	NS	−4.8	−4.8
C6:0	1.5	1.4	1.4	0.1	***	NS	−6.7	−6.7
C8:0	1.0	0.9	0.9	0.1	*	NS	−10.0	−10.0
C10:0	2.4	2.2	2.1	0.3	*	NS	−8.3	−12.5
C12:0	2.9	2.6	2.5	0.4	**	NS	−10.3	−13.8
C14:0	11.3	10.4	9.9	0.7	***	*	−8.0	−12.4
C16:0	32.8	28.8	27.3	2.1	***	†	−12.2	−16.8
C18:0	11.1	13.5	13.9	1.2	***	NS	21.6	25.2
cis-9 C18:1	18.8	20.6	22.1	1.6	***	**	9.6	17.6
trans-10 C18:1	0.2	0.2	0.2	0.1	NS	NS	0.0	0.0
trans-11 C18:1	1.7	2.2	1.9	0.5	**	†	29.4	11.8
C18:2n−6	1.7	1.4	1.6	0.3	**	*	−17.6	−5.9
C18:3n−3	0.7	0.9	1.0	0.2	***	*	28.6	42.9
ΣCLA	0.8	1.1	0.9	0.2	**	†	37.5	12.5
ΣSFA	67.7	64.4	62.6	2.3	37.5	12.5	−4.8	−7.5
ΣMUFA	22.34	24	25.7	1.7	**	**	7.4	14.9
ΣPUFA	2.81	2.75	3.03	0.4	NS	*	−2.1	7.8
ΣFA ≤ C16:0	59.3	53.3	51.0	2.9	***	*	−10.1	−14.0

ΣCLA: CLA 9c,11t; CLA 10t,12c; CLA c,c; CLA t,t; ΣFA ≤C16: sum of 27 FA from C4:0 to C16:1 11c. FA proportions are expressed as percents of total fatty acids. RMSE = Root mean square error; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.
NS = $p > 0.10$; † $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[12], we calculated this (expressed in g/cow/day) from C16:0 (equation 1: −15%; $p < 0.001$) and also from C8:0 to C16:0 (equation 2: −14%; $p < 0.001$; table 4). The other estimation of methane output by Weill et al. [30] (equation 3, expressed in g/l of milk) also estimated a significant decrease (−10%, $p < 0.001$).

Moreover, the feed form of flax has a significant effect on methane output as estimated by Chilliard et al. [12] with equation 1 (FM: 406 g/day/cow, EF: 382 g/day/cow; $p < 0.01$) and with equation 2 (FM: 367 g/day/cow, EF: 345 g/day/cow; $p < 0.01$). The prediction of methane output by equation 3 shows a significant decrease from

Table 4. Effects of flaxseed supplementation in experimental diet of dairy cows on methane output.

	Control diet	Flax diet		Statistics		
		flax meal	extruded flax	RMSE	flax effect	feed form effect of flax
Methane output in g/day/cow						
Calculated from C16:0 (equation 1)	474	406	382	26.5	***	**
Calculated from C8:0 to C16:0 (equation 2)	421	367	345	32.6	***	**
Methane output in g/l of milk						
Calculated from FA and yield (equation 3)	13.6	12.2	11.6	0.8	***	***

RMSE = Root mean square error. ** $p < 0.01$; *** $p < 0.001$.

13.6 to 12.2 and 11.6 g/l of milk, for FM and EF diets, respectively.

Discussion

Milk Fatty Acid Composition

Results of the present study indicate that dietary flaxseed supplementation decreased C16:0 (and to a lesser extent other short and medium chain SFA) and increased C18:3n–3 levels in milk. This is consistent with previous studies [31–33]. Different ratios that characterize milk nutritional properties such as Σ_{UFA} (unsaturated FA)/Σ_{SFA}, trans-11 C18:1n–9/trans-10 C18:1n–9 or C18:2n–6/C18:3n–3 were strongly improved. It is interesting to note that these changes occurred with conserved grass-based diets, and that milk FA composition already appeared nutritionally interesting during the control period, when compared to standard winter FA profiles of milk.

A number of studies have reported that the feed form of flax (whole seed, ground seed, extruded, oil) were all efficient in improving the nutritional quality of milk [32–36]. However, the efficiency of FA deposition in milk is highly dependent of the bioavailability of fat in seed and to the biohydrogenation process in rumen [37–39]. Consequently, the form of dietary flax that is fed could have huge effects on the magnitude of milk FA improvements. Chesneau et al. [39] reported that extruded flaxseed improved the availability of fat. Extrusion also protects fat from ruminal biohydrogenation and thus influences the efficiency of transfer of FAs into the milk and, among other things, determines the degree of n–3 FA enhancement in milk as reported by Gonthier et al. [40]. Moallem [31] supposed that heat treatment, such as extrusion, might encapsulate or tie up the FA fraction of seeds. This could lead to a better efficiency of milk improvement with extruded linseed by comparison with other forms of feed, as described by the present study. Indeed, the improvement of milk FA was better in the EF groups than in the FM groups.

Methane Output

Milk production is often criticized for its high carbon footprint [3, 8, 41], which is especially linked to the enteric methane output of the cow. However, it is possible to reduce the methane output using different feeds. The present paper highlighted that methanogenesis and lipogenesis (de novo SFA) are physiologically linked through volatile

FA production pathways. When rumen fermentation produces C2, this generates hydrogen release and hydrogen is used by methanogenic bacteria to produce methane [2, 6]. C2 is used in the mammary epithelium cell for the de novo synthesis of SFA from C4 to C16 [42]. So, high C2 production is linked on the one hand to high methane production and on the other hand to high atherogenic SFA production. This relationship was reported by Chilliard et al. [12] who found that cows producing milk with high levels of C16:0 also have high levels of methane output.

Some researchers have evaluated the effects of lipid supplementations on both milk FA profile and methanogenesis. Trials with C18:3n–3 supplementation (in the form of flaxseed) have exhibited a strong link between the C16:0 content in milk and methane output [12, 13]. However, trials with other sources of lipids rich in C18:2n–6, like soybean or cottonseed [43, 44], or in C12:0 and C14:0 [45] have exhibited decreases in C16:0 in milk content without effects on methane output. Consequently, the positive relationship between C16:0 content in milk and methane output may only be validated with C18:3n–3-rich diets, probably because the effects were due to isomers formed from the biohydrogenation of dietary C18:3n–3 by the methanogenic rumen flora. This effect does not exist, or only to a lesser extent, with diets where C18:3n–3 is not the predominant FA in the cows' diet.

Animal and Human Health Benefits of a High Nutritional Value of Milk

There are different possible ways to decrease de novo SFA production through changes in the diets of dairy cows other than high levels of supplementary C18:3n–3 [17].

Large decreases in de novo SFA can be achieved through different methods. Some methods linked with acidosis and 'low-fat syndrome', like high-grain or high-starch diets, decreased de novo SFA content in milk and were considered as deleterious [46–49]. In some cases, such practices could drastically increase the concentration of trans FA isomers other than trans-11 C18:1. These other (than trans-11) trans isomers are usually considered as deleterious for men's health [50, 51], while some beneficial FA and especially short-chain FA decreased. Generally, in most of the studies, a decrease of milk's SFA induced a parallel increase of trans FA and especially trans-10 C18:1 or other isomers than trans-11 C18:1 [46, 52–54].

The modification of milk FA using flax supplementation can be linked with improvements in human nutrition. Indeed, Ulbricht and Southgate [55] suggested that high levels of C12:0, C14:0 and C16:0 in human diets are correlated with a higher prevalence of cardiovascular problems. In addition to milk FA profiles exhibiting mild decreases in C16:0 and mild increases in C18:3n–3, improvements in cardiovascular markers are also found [51], but these effects disappear with low C16:0 and high trans FA contents in milk. In human trials, without changing the level of consumption of dairy products, it was possible to improve the FA profile of red blood cells [56], insulin resistance in diabetic volunteers [57] and anthropometric markers in overweight volunteers [58] by consuming products from cows fed supplementary extruded flaxseed.

A positive link between C16:0 content in milk and improvement of human health markers was shown for mild decreases of C16:0 and other de novo issued FA, but this link was not shown when strong decreases of C16:0 were linked with increases of trans C18:1 isomers other than trans-11 [51].

Conclusion

In the present trial, a grass-based diet supplemented with flaxseed, simultaneously improved the milk lipid profile and reduced methane output, leading to beneficial effects on animal health, human health and environment. The economic and technical effects of the trial diets (data not shown) indicate that this method is economically and logistically feasible to implement. Moreover, compared to traditional high-corn, high-soybean diets, grass and flaxseed are also interesting crops in terms of environmental impact, as they only need low input in fields.

The present study highlighted that with a high dietary C18:3n–3 content from grass and flaxseed in dairy cow diet, the ratio between C16:0 and C18:3n–3 in milk was a useful indicator. Indeed, its decrease was linked not only to a drop in C16:0, but also to the way in which this drop was obtained. This ratio of C16:0/C18:3n–3 could be used as a good marker of the nutritional and environmental value of milk.

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