EFFECTS OF LINSEED LIPID ON RUMINAL FATTY ACID METABOLISM, NUTRIENT DIGESTIBILITY, MILK FATTY ACID COMPOSITION, AND METHANE EMISSION IN RUMINANTS

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Received: September 19, 2012; Revised: February 26, 2013; Accepted: March 04, 2013

Abstract

Linseed lipid in the dietary composition is a major factor influencing the fatty acid composition of milk from ruminants, because fatty acids which reach the duodenum are, at least in part, of dietary origin as well as the result of ruminal microbial biohydrogenation of dietary lipids. In this review, the effect of linseed lipid on ruminal fatty acid metabolism, nutrient digestibility, microbial protein synthesis, milk fatty acid composition, and methane emission are discussed. The studies that are undertaken on ruminants mainly use diets supplemented with different linseed forms like linseed oil, extruded linseeds, and rolled linseeds, as sources of n-3 fatty acids. The use of linseed lipid generally increased the flow of *cis* 18:1, *trans* 18:1, CLA, 18:3n-3, and total unsaturated fatty acids at the duodenum. Ruminants fed diets supplemented with linseed lipid had greater milk n-3 fatty acids and a sum of unsaturated fatty acids, and lower ruminal methane production. However, a linseed lipid supplement may have some negative effects on nutrient digestibility, microbial protein synthesis, and milk yield. The conclusion is that linseed lipid has a high potential to increase milk fatty acid quality and mitigate methane emission in ruminant feeding.

Keywords: Linseed lipid, methane emission, milk fatty acid, ruminant

Introduction

Public health guidelines in most developed countries have recommended population-wide decreases in saturated and *trans* fatty acids (FA), with an increase in 18:3n-3, 20:5n-3, and 22:6n-3 in the human food chain to reduce the incidence of chronic diseases (WHO, 2003). Dietary consumption of omega-3 fatty acids (n-3 FA) is beneficial for human health (Gebauer *et al*., 2006), and conjugated linoleic acid (CLA) from ruminant fat has been shown to exert anti-carcinogenic benefits in experimental animal models (Huth *et al*., 2006). There is growing interest in elevating n-3 FA and CLA contents in ruminant products, and supplementation of ruminant diets with oilseeds rich in α-linolenic acid has been

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shown to increase n-3 FA and CLA contents in meat and milk. For example, including linseed, an oilseed rich in n-3 PUFA (polyunsaturated fatty acids) (Mach *et al*., 2006), or pasture grass (Noci *et al*., 2005) in diets of beef cattle for an extended period of time increased n-3 FA and CLA levels in their meat. Adding linseed to diets of dairy cows increased the α-linolenic acid contents and CLA in milk fat (Chilliard *et al*., 2009; Caroprese *et al*., 2010).

Production of greenhouse gases (GHG) from livestock and their impact on climate change is a worldwide concern (Steinfeld *et al*., 2006). It has been reported that enteric methane (CH_4) is one of the most important anthropogenic GHG emitted at the farm level in ruminant production systems. It is the main contributor to livestock GHG ranging from 48 to 65% in bovine milk production systems and from 56 to 65% in New Zealand dairy farms (Basset-Mens *et al*., 2009). Similarly, in meat production systems in France, enteric CH_4 contributed 58-66% of GHG emitted on farms (Veysset *et al*., 2010). Moreover, energy loss from animals due to CH_4 production ranges from 2 to 12% of gross energy (GE) intake in mature cattle (Johnson and Johnson, 1995). Thus, the development of feeding strategies to mitigate these CH_4 emissions may not only be environmentally friendly for the planet but also may bring nutritional benefits for the animal. Incorporating oilseeds to the diets of cattle has been shown to reduce enteric CH_4 emissions (Beauchemin *et al*., 2008; Martin *et al*., 2010).

The enteric CH_4 suppressing effects of linseed may partially be due to the negative effects that high intake levels of lipids can have on fibre digestibility (Martin *et al*., 2008; Beauchemin *et al*., 2009), a response that would be undesirable in terms of feed utilization efficiency and on dry matter intake. Further evaluation is needed to establish the conditions whereby linseed lipid can be added to cattle diets to reduce enteric CH_4 production without decreasing digestibility and animal performance.

Therefore, the aims of the current review are to summarize the effects of linseed lipid on ruminal fatty acid metabolism, nutrient digestibility, microbial protein synthesis, milk fatty acid composition, methane emission, and milk yield in ruminants.

Fatty Acid Composition of Linseed Oil

Shingfield *et al*. (2011) reported thatlinseed oil is rich in polyunsaturated fatty acids, particularly α-linolenic acid (57.8%), the essential omega-3 fatty acid, and linoleic acid (15.8%), the essential omega-6 fatty acid (Table 1). These 2 fatty acids cannot be synthesized in humans and other mammals; they must be obtained from dietary fats and oils and are therefore defined as essential fatty acids. Yalcin *et al*. (2012) reported that α-linolenic acid content is less than 7% in other common plant sources, such as canola oil, corn oil, cottonseed oil, olive oil, sunflower oil, and soybean oil. In rumen, α-linolenic acid is a precursor to synthesize eicosapentaenoic acid and docosahexaenoic acid (Conklin *et al*., 2010), meanwhile linoleic acid is converted to *cis*-9, *trans*-11 CLA, and *trans*-10, *cis*-12 CLA (Bauman and Griinari, 2003; Collomba *et al*., 2006). The *cis*-9, *trans*-11 CLA contributes to 75–90% of total CLA in the milk, while *trans*-10, *cis*-12 CLA can cause milk fat depression (Bauman and Griinari, 2003).

Effect of Linseed Lipid on Ruminal Fatty Acid Metabolism

Several studies have examined the potential of fish oil (FO) (Shingfield *et al*., 2003; Kim *et al*., 2008; Lee *et al*., 2008), linseed oil (LSO) (Loor *et al*., 2004; Doreau *et al*., 2009b), or linseeds (Scollan *et al*., 2001; Doreau *et al*., 2009b) in the diet to alter the supply of n-3 PUFA available for absorption in cattle. Furthermore, the effects of supplementing the diet with LSO and FO on the flow of FA at the duodenum have been

examined in steers (Scollan *et al*., 2001) and sheep (Wachira *et al*., 2000; Chikunya *et al*., 2004), but there are no reports on the impact of feeding a combination of FO and LSO on the ruminal lipid metabolism in growing cattle. Therefore, a study to investigate the effect of LSO and FO alone or as an equal mixture on the ruminal FA metabolism in growing steers fed maize silage-based diets was conducted by Shingfield *et al*. (2011) (Table 2). The dietary lipid supplement had no effect on the flow of dry matter (DM), organic

Table 1. Fatty acid composition of linseed oil (g/100g of total FA) (Shingfield *et al*., 2011)

Fatty acids		Fatty acids	
12:0 (lauric)	ND	20:2n-3 (auricolic)	ND
14:0 (myristic)	0.03	20:2n-6 (eicosadienoic)	0.01
14:1 *cis*-9 (myristoleic)	ND	20:2n-9 (dihomotaxoleic)	ND
15:0 (pentadecanoic)	0.02	20:3n-6 (dihomo-ɣ-linolenic acid, DHGLA)	ND
16:0 (palmitic)	4.23	20:4n-3 (juniperonic)	ND
16:1 *cis*-9 (palmitoleic)	ND	20:4n-6 (arachidonic, AA)	ND
16:2n-4	ND	20:5n-3 (eicosapentaenoic, EPA)	ND
16:4n-1	ND	21:5n-3	ND
16:4n-3	ND	22:0 (behenic)	0.12
17:0 (heptadecanoic)	0.06	22:1 *cis*-11 (cetoleic)	ND
18:0 (stearic)	2.74	22:1 *cis*-13 (erucic)	0.01
18:1 *cis*-9 (oleic, OA)	16.5	22:5n-3 (docosapentaenoic, DPA)	ND
18:1 *cis*-11 (vaccenic)	0.62	22:5n-6 (osbond)	ND
18:1 *cis*-12	ND	22:6n-3 (docosahexaenoic, DHA)	ND
18:1 *trans*[1]	ND	24:0 (lignoceric)	0.08
18:2 *trans*[2]	0.05	24:1 *cis*-15 (nervonic)	0.01
18:2n-4 (densipolic)	ND	26:0 (cerotic)	0.03
18:2n-6 (linoleic, LA)	15.8	28:0 (montanic)	ND
18:2n-9 (taxoleic)	ND	Other	0.16
18:3n-3 (α-linolenic, ALA)	57.8	SFA	7.55
18:3n-6 (ɣ-linolenic, GLA)	ND	MUFA	17.4
18:4n-3 (stearidonic, SDA)	ND	PUFA	73.7
20:0 (arachidic)	0.12	n-6 PUFA	15.9
20:1 *cis*-9 (gadoleic)	0.04	n-3 PUFA	57.8
20:1 *cis*-11 (gondoic)	0.19	PUFA n-6/n-3	0.27
20:1 *cis*-13 (paullinic)	ND	FA, g/kg DM	953

FA: fatty acids; ND: not detectable. [1]: Sum of trans-9 18:1, trans-10 18:1, and trans-11 18:1. [2]: Sum of cis-9, trans-12 18:2, trans-9, cis-12 18:2, and trans-9, trans-12 18:2. SFA: sum of all even chain fatty acid up to 22:0. MUFA: sum of 14:1, 16:1, 18:1, 20:1, 22:1, and 24.1. PUFA: sum of 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:5, and 22:6. n-6 PUFA: sum of 18:2, 18:3n-6, 20:2, 20:3n-6, and 20:4. n-3 PUFA: sum of 18:3n-3, 20:5, 22:5, and 22:6. PUFA n-6/n-3 = C18:2n-6/C18:3n-3.

matter (OM), neutral detergent fibre (NDF), or starch at the duodenum. The inclusion of LSO in the diet increased 16:0, 3, 7, 11, 15-tetramethyl 16:0, 18:0, *cis* 18:1, *trans* 8:1, CLA, and 18:3n-3 at the duodenum, whereas FO increased the flow of 14:0, 15:0, 15:0 iso, 16:0, 3, 7, 11, 15-tetramethyl 16:0, total 16:1, 17:0, *cis* 18:1, *trans* 18:1, 20- (with the exception of 20:0 and 20:4n-6), and 22- (other than 22:0) carbon fatty acids, but decreased 18:0 at the duodenum (Table 2). Alterations in the flow of FA on the fish oil and linseed oil (FOLSO) diet were in most cases intermediate of those to FO and LSO. Similar results were found in the research of Doreau *et al*. (2009a) that dry cows offered 4.9% DM LSO and 1.6% DM linseed meal had a greater duodenal flow of 18:0, *cis* 18:1, *trans* 18:1, CLA, and total FA than the control diet.

Table 2. Effect of linseed oil and fish oil in the diet on the flow of nutrients at the duodenum in growing steers (Shingfield *et al*., 2011)

Flow, g/d	Treatments[1]				SEM	P
	Control	LSO	FO	FOLSO		
DM	5389	5688	5142	5584	193.2	0.30
OM	4483	4749	4360	4585	186.9	0.55
NDF	1484	1546	1448	1587	73.1	0.58
Starch	472	430	445	482	60.6	0.92
N	224	218	188	204	8.0	0.08
Non-ammonia N	219^{a}	213^{a}	182^{b}	199^{ab}	7.6	0.05
12:0	0.48	0.50	0.53	0.50	0.05	0.93
13:0	0.12	0.13	0.14	0.15	0.013	0.37
14:0	2.11^{c}	2.27^{c}	9.33^{a}	6.28^{b}	0.451	<0.001
15:0	2.59^{c}	2.97^{bc}	4.09^{a}	3.75^{ab}	0.226	0.011
15:0 iso	1.47^{b}	1.59^{b}	2.00^{a}	1.71^{ab}	0.097	0.039
15:0 anteiso	3.01	3.30	3.87	3.36	0.213	0.13
16:0	39.1^{c}	49.9^{b}	84.2^{a}	74.8^{a}	3.01	0.001
16:0 iso	1.16	1.66	0.93	1.73	0.277	0.22
3, 7, 11, 15-tetramethyl-16:0	0.27^{c}	0.36^{b}	0.48^{a}	0.47^{a}	0.026	0.004
16:1 total	0.67^{c}	0.81^{c}	10.9^{a}	5.71^{b}	0.892	<0.001
17:0	1.75^{b}	1.98^{b}	3.11^{a}	2.69^{a}	0.161	0.003
17:0 iso	0.68	0.71	1.00	1.06	0.104	0.09
17:0 anteiso	1.39	1.48	1.89	2.06	0.182	0.11
17:1 *cis*-8	0.0^{c}	0.0^{c}	0.14^{a}	0.08^{b}	0.010	0.001
18:0	157^{b}	273^{a}	51.4^{d}	123^{c}	9.04	<0.001
18:1 total *cis*	17.9^{b}	31.0^{a}	28.9^{a}	31.1^{a}	1.17	<0.001

Table 2. Effect of linseed oil and fish oil in the diet on the flow of nutrients at the duodenum in growing steers (Shingfield *et al*., 2011) (continute)

Flow, g/d	Treatments[1]				SEM	P
	Control	LSO	FO	FOLSO		
18:1 total *trans*	24.5^{c}	84.4^{b}	108^{ab}	125^{a}	11.2	0.002
18:1 total	42.4^{c}	115^{b}	137^{ab}	156^{a}	12.0	0.003
CLA total	0.38^{b}	3.18^{a}	0.27^{b}	0.55^{b}	0.368	0.004
18:3n-3	1.57^{bc}	4.36^{a}	1.23^{c}	2.29^{b}	0.217	<0.001
20:0	2.68^{b}	3.22^{b}	2.86^{b}	5.62^{a}	0.351	0.003
20:1 *cis*-11	0.29^{b}	0.42^{b}	3.32^{a}	2.72^{a}	0.192	<0.001
20:2n-6	0.06^{c}	0.08^{c}	0.83^{a}	0.35^{b}	0.055	<0.001
20:3n-3	0.04^{b}	0.04^{b}	5.14^{a}	0.86^{b}	0.414	<0.001
20:3n-6	0.05^{b}	0.10^{b}	0.49^{a}	0.17^{b}	0.053	0.004
20:4n-6	0.09	0.20	0.11	0.08	0.040	0.24
20:5n-3	0.11^{b}	0.16^{b}	1.25^{a}	0.93^{a}	0.120	0.001
22:0	1.35^{c}	1.68^{b}	1.37^{c}	2.03^{a}	0.055	<0.001
22:1 *cis*-13	0.18^{c}	0.21^{c}	0.61^{a}	0.40^{b}	0.039	<0.001
22:2n-6	0.02^{b}	0.02^{b}	0.13^{a}	0.11^{a}	0.012	<0.001
22:4n-6	0.20^{b}	0.17^{b}	0.32^{a}	0.16^{b}	0.023	0.008
22:5n-3	0.15^{c}	0.17^{c}	2.62^{a}	0.80^{b}	0.175	<0.001
22:6n-3	0.07^{b}	0.08^{b}	0.97^{a}	0.78^{a}	0.117	0.003
23:0	0.29^{b}	0.31^{ab}	0.36^{a}	0.38^{a}	0.018	0.046
24:0	1.75	1.93	1.62	1.85	0.106	0.29
Total SFA	217^{b}	347^{a}	170^{c}	232^{b}	7.4	<0.001
Total MUFA	43.6^{c}	117^{b}	153^{ab}	165^{a}	12.5	0.002
Total PUFA	17.0^{b}	31.1^{a}	31.3^{a}	30.5^{a}	1.01	<0.001
Total n-3 PUFA	1.94^{c}	4.80^{b}	11.2^{a}	5.65^{b}	0.62	<0.001
Total fatty acids	278^{d}	495^{a}	354^{c}	428^{b}	12.3	<0.001

$^{a–d}$ Within a row, means without a common superscript differ (P<0.05). [1] Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LSO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (FOLSO). DM: dry matter; OM: organic matter, NDF: neutral detergent fibre; N: nitrogen; CLA: conjugated linoleic acid; SFA: short chain fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Previous studies have shown that LSO tends to decrease the net lipid balance in the rumen and at greater amounts can result in the fatty acid flow at the duodenum being less than the dietary intake (Loor *et al*., 2004; Doreau *et al*., 2009a). Supplementing maize silage-based diets with FO and FOLSO increased the duodenal flow of 20:5n-3 and 22:6n-3 at the duodenum, but this was marginal relative to the intake, indicating that these fatty acids were extensively hydrogenated in the rumen (Shingfield *et al*., 2011). An LSO-supplemented diet increased 18:3n-3 biohydrogenation in the rumen, while no significant difference was observed in the diet supplemented with FO alone.

Effect of Linseed Lipid on Nutrient Digestibility and Microbial Protein Synthesis

A major concern in using linseed in animal feeding is the potential negative effects of FA from linseed on ruminal digestion. Several experiments in the 1980s strongly showed negative effects of linseed supplemented at 50–70 g/kg DM diet on ruminal digestion in sheep (Ikwuegbu and Sutton, 1982; Sutton *et al*., 1983). This effect was ascribed partly to a large drop in the protozoa population and partly to a shift of volatile fatty acid (VFA) composition towards propionate. To better understand the effect of linseed in different forms on nutrient digestibility in ruminants, a study was conducted on dry Holstein cows to investigate the effects of rolled linseeds (RLS, 75 g/kg DM), extruded linseeds (ELS, 75 g/kg DM), and a linseed oil and linseed meal mixture supplied at 26 g/kg DM and 49 g/kg DM, respectively (LSOM) (Doreau *et al*., 2009a). The results were that the supplementation of linseed in different forms had no effects on nutrient digestibility (Table 3), nitrogen retention, ruminal digestion, and microbial protein synthesis (Table 4) of dry Holstein cows. Sutter *et al*. (1999) and Machmüller *et al*. (2000) reported that steers and bulls receiving 20-30 g/kg diet DM oil from linseeds did not find any decrease in OM and NDF digestibility. Similarly, Wachira *et al*. (2000) and Ueda *et al*. (2003) did not show any difference on whole tract OM and fibre digestibility with a supplement of 30 g/kg DM LSO in lactating cows. Other experiments with sheep or cattle fed linseeds did not show decreases in total tract OM digestibility (Wachira *et al*., 2000; Petit *et al*., 2002). Total tract and fore-stomach digestibility did not modify in dry cows supplemented at 75 g/kg DM rolled linseeds (or 75 g/kg DM extruded linseeds, or 26 g/kg DM linseed oil plus 49 g/kg DM linseed meal) (Doreau *et al*., 2009a); however, Martin *et al*. (2008) observed a decrease in total tract digestibility with a supply of 5% oil from linseed fed as crude, extruded, or free oil in dairy cows. These results concluded that the effects of LSO may depend on the form of the linseed, level of inclusion, and/or the level of feeding (i.e., at maintenance, the negative effects of linseed on digestion can be higher than in high-producing animals due to a longer retention time of digesta in the rumen at low feed intake), which are higher in cows than in sheep and suggest that LSO may have a more depressing effect on digestibility than other linseed forms (Doreau *et al*., 2009a). The form of the supply of linseed lipids in the diet did not modify the duodenal flow of N (Table 4), which is consistent with the general effects of lipids on ruminal N metabolism (Doreau and Ferlay, 1995). Rolled and extruded linseed protein was not protected against microbial degradation (Gonthier *et al*., 2004), as it has been shown that extrusion limits ruminal CP degradation, especially due to its temperature effect (Poncet *et al*., 2003). This may explain the similar result in the microbial nitrogen in cows fed linseed in different forms (Table 4).

Effect of Linseed Lipid on Milk Fatty Acid Composition

LSO is a potential alternative to FO as a dietary source of unsaturated fatty acids. There is increasing interest in adding LSO as an alternative to FO to dairy cow diets because

of its FA profile; LSO α-linolenic acid contributes dietary n-3 FA and promotes an increased CLA content of milk from ruminants (Chilliard *et al*., 2007). The effect of supplementing a basal dairy cow diet containing 800 g of saturated animal fat (Control) at a level of 200 g FO and 600 g LSO per day (FOLSO) on the milk FA was investigated by Brown *et al*. (2008) (Table 5). The concentration of milk *cis*-9, *trans*-11 CLA was higher in the FOLSO diet (2.56% of total FA and 16.4 g/d, respectively) than the control diet (0.66% of total FA and 6.44 g/d, respectively). The concentrations of milk *trans*-C18:1 and vaccenic acid were higher with the FOLSO diet (13.5 and 7.48% of total FA, respectively) than the control diet (3.69 and 2.27% of total FA, respectively), an observation in agreement with Bernard *et al*. (2009) relating to the dairy goat fed diet based on natural grassland hay or maize silage supplemented with LSO at 130 g/d. Dairy cattle offered whole linseed at 72 g/kg DM had higher milk *cis*-9 C18:1, C18:3n-3, MUFA, and PUFA than the animals fed the control diet (Petit and Côrtes, 2010). Similar results were also found for the dairy cows supplemented with extruded linseed at

Table 3. Intake, total tract apparent digestibility, and ruminal and intestinal organic matter digestibility in cows fed linseed in different formsa (Doreau *et al*., 2009a)

	Diets[2]				SEM
	Control	RLS	ELS	LSOM	
DM intake (kg/d)	10.5[1]	10.0	9.9	9.9	0.24
Total tract apparent digestibility					
DM	0.695	0.700	0.696	0.704	0.008
OM	0.718	0.720	0.718	0.724	0.007
NDF	0.527	0.558	0.541	0.525	0.012
ADF	0.448	0.486	0.471	0.452	0.015
Forestomach OM digestibility					
g/kg OM intake	533	598	518	517	36.4
g/kg OM totally digested	742	830	721	715	47.7
Intestinal OM digestibility					
g/kg OM intake	185	122	200	206	34.9
g/kg OM totally digested	258	170	279	285	47.7
Forestomach NDF digestibility					
g/kg NDF intake	400	476	456	419	28.4
g/kg NDF totally digested	759	851	845	800	55.1
Intestinal NDF digestibility					
g/kg NDF intake	127	82	85	106	29.3
g/kg NDF totally digested	241	149	155	200	54.9

[1] *For all variables, differences among diets were not significant (i.e., P> 0.05).*

[2] *RLS: rolled linseeds; ELS: extruded linseeds; LSOM: linseed oil + linseed meal. DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre.*

28 g/kg DM (Moallem, 2009). Fuentes *et al*. (2008) reported that dairy cows supplemented with 5.5% DM extruded linseed for 90d had greater C18:1 *cis* 9, CLA, ω3, MUFA, and PUFA, compared to the control diet. Kennelly (1996) noted that physical processing of oilseeds increased the overall lipid digestibility of the oilseeds and enhanced their effect on milk FA composition over the intact seed. Glasser *et al*. (2008) concluded that the content of total C18 fatty acids in milk fat was quadratically increased by all oilseed lipid supplements according to added lipid, that most supplements resulted in significant increases in *cis* and *trans* C18:1, and that physical protection of the supplement greatly improved the linoleic acid and α-linolenic acid contents of milk fat. These may relate to the optimization of mammary gland desaturase activity through the supply of C18 fatty acids, whether directly from the diet or as a product of rumen biohydrogenation (Woods and Fearon, 2009).

Effect of Linseed Lipid on Methane Emission

Dietary fatty acids, particularly PUFA, are among the most promising dietary alternatives able to depress ruminalmethanogenesis (Martin *et al*., 2006). Linseed polyunsaturated FAs decrease methane production through a toxic effect on microorganisms involved in fibre digestion and hydrogen production such as protozoa and cellulolytic bacteria (Martin *et al*., 2010). This effect, observed with all long-chain PUFA, is probably through an action on the cell membrane particularly of gram-positive bacteria. It has been shown *in vitro* that α-linolenic acid (the predominant FA in linseed oil) is particularly toxic for the 3 cellulolytic bacterial species

Table 4. Nitrogen balance, ruminal N digestion, and microbial protein synthesis in cows fed linseed in different formsa (Doreau *et al*., 2009a)

	Diets[2]				SEM
	Control	RLS	ELS	LSOM	
N intake (g/d)	243[1]	238	235	236	1.2
N in feces (g/d)	76	75	70	72	1.9
N in urine (g/d)	136	142	142	132	5.3
N retained (g/d)	31	21	22	33	7.3
Duodenal NAN[3] (g/d)	204	171	176	188	9.2
Microbial (g/d)	107	82	92	94	5.3
Non-microbial (g/d)	97	89	84	94	9.5
Duodenal NAN (g/kg N intake)	854	721	757	800	40.5
Microbial N (g/kg DM intake)	104	81	93	97	5.0
Microbial N (g/kg OMDR[4])	22.2	14.5	20.1	20.2	1.60
Non-microbial N (g/kg DM intake)	9.2	9.0	8.5	9.5	0.76
N intestinal disappearance	0.630	0.559	0.591	0.620	0.031

N: nitrogen. [1] For all variables, differences among diets were not signi?cant (i.e., P > 0.05). [2] RLS: rolled linseeds; ELS: extruded linseeds; LSOM: linseed oil + linseed meal. [3] NAN: non-ammonia N. [4] OMDR: OM apparently digested in the rumen.

(Fibrobactersuccinogenes, Ruminococcusalbus, and *Ruminococcusflavefaciens),* because it disrupts cell integrity (Maia *et al*., 2006). In addition, a direct toxic effect of PUFA on methanogens that use hydrogen for methane production may have occurred, as shown in vitro with linseed oil hydrolysate (Prins *et al*., 1972). In this case, free hydrogen may

Table 5. Milk fatty acid composition in dairy cows fed linseed oil and fish oil mixing (Brown *et al*., 2008)

Fatty acids	Treatments		SEM	P
	Control	FOLSO		
C4:0	1.43	0.91	0.08	0.01
C6:0	1.24	0.68	0.07	0.01
C8:0	0.77	0.38	0.05	0.01
C10:0	1.57	0.76	0.11	0.01
C12:0	1.85	1.08	0.09	0.01
C14:0	7.78	5.76	0.17	0.01
C16:0	27.4	19.9	0.59	0.01
C17:0	0.87	0.48	0.03	0.01
C18:0	15.6	9.45	0.94	0.01
C18:1 *trans*	3.69	13.5	0.89	0.01
trans-6/8	0.29	0.72	0.05	0.01
trans-9	0.25	0.73	0.06	0.01
trans-10	0.23	2.09	0.47	0.02
trans-11, VA	2.27	7.48	0.76	0.04
trans-12	0.40	1.46	0.13	0.01
trans-16	0.23	1.03	0.06	0.01
C18:1 *cis*-9	24.0	22.4	1.19	0.37
C18:2 *trans*-11, *cis*-15	0.22	2.09	0.12	0.01
C18:2 *cis*-9, *cis*-12	1.99	1.66	0.13	0.09
C18:3n-3	0.56	1.26	0.08	0.01
CLA *cis*-9, *trans*-11	0.66	2.56	0.28	0.05
CLA *trans, trans*	0.10	0.38	0.06	0.03
C20:5n-3, EPA	0.03	0.07	<0.01	0.01
C22:4n-6	0.03	0.04	<0.001	0.04
C22:5n-3	0.05	0.07	<0.001	0.01
C22:6n-3, DHA	0.01	0.06	<0.001	0.01
SMCFA[1]	43.7	30.6	1.04	0.01
SFA[2]	59.4	40.8	0.99	0.01
UFA[3]	34.8	47.9	0.99	0.01
Unknown	5.73	11.2	1.76	0.01

VA: vaccenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. [1] SMCFA: sum of short and medium chain fatty acids (C4:0-C16:0). [2] SFA: sum of saturated fatty acids. [3] UFA: sum of unsaturated fatty acids.

accumulate in the gas mixture, resulting in growth inhibition of cellulolytic bacteria (Wolin *et al*., 1997). It has been shown that FA from linseed can decrease CH_4 production in vitro (Broudiscou and Lassalas, 1991), as well as in vivo in sheep at maintenance (Czerkawski *et al*., 1966), and in growing lambs (Machmüller *et al*., 2000). However, this effect has been scarcely confirmed in dairy cows. Therefore, a study to investigate

Table 6. Methane emission in lactating dairy cows fed diets supplemented with linseed in different forms (Martin *et al*., 2008)

Item	Diets[1]				SEM	P
	Control	CLS	ELS	LSO		
CH_4, g/d	418[a]	369[b]	258[c]	149[d]	13.6	<0.001
CH_4, % GE intake	6.7[a]	5.7[b]	4.8[c]	3.0[d]	0.21	<0.001
CH_4, g/kg of OM intake	22.0[a]	19.8[b]	16.3[c]	10.5[d]	0.72	<0.001
CH_4, g/kg of NDF intake	63.8[a]	59.3[a]	50.7[b]	27.5[c]	2.19	<0.001
CH_4, g/kg of digested OM	31.4[a]	30.2[a]	24.5[b]	16.2[c]	1.08	<0.001
CH_4, g/kg of digested NDF	136[a]	141[a]	136[a]	68.1[b]	6.42	<0.001
CH_4, g/kg of milk	17.4[a]	17.9[a]	12.2[b]	8.1[c]	0.94	<0.001
CH_4, g/kg of 4% fat-corrected milk	19.3[a]	16.4[ab]	14.8[b]	9.3[c]	1.27	<0.001
CH_4, % milk energy output	33.8a[a]	29.0[a]	25.7[a]	15.7[b]	2.30	<0.001

[1] *CLS: crude linseeds; ELS: extruded linseeds; LSO: linseed oil. CH_4: methane; GE: gross energy; OM: organic matter; NDF: neutral detergent fibre.*

Table 7. Effect of linseed lipid on feed intake and milk yield of dairy cows

References	Animals	Supplement	DMI, kg/d	MY, kg/d
Fuentes *et al*., 2008	Holstein cows	Control	24.6	45.6
		ELS at 5.5% DM	23.9	44.5
		SEM	1.90	0.68
Moallem, 2009	Israeli-Holstein cows	Control	-	44.2[a]
		EFS at 4% DM	-	45.4[b]
		SEM	-	0.09
Martin *et al*., 2008	Holstein cows	Control	19.8[a]	23.0[a]
		CLS at 5.7% DM	19.5[a]	21.5[a]
		ELS at 5.7% DM	16.7[b]	20.8[ab]
		LSO at 5.7% DM	14.7[c]	18.9[b]
		SEM	0.30	0.71

[a–c] *Within a column, means without a common superscript differ ($P < 0.05$). DM: dry matter; DMI: dry matter intake; MY: milk yield; ELS: extruded linseeds; EFS: extruded flaxseeds (linseeds); CLS: crude linseeds; LSO: linseed oil*

the CH_4 output in response to feeding dairy cows with crude linseed (CLS), extruded linseed (ELS), or linseed oil (LSO) had been conducted by Martin *et al.* (2008) (Table 6). The lower daily CH_4 output (g/d), CH_4 output per OM intake (g/kg), and CH_4 output per GE intake (%) were found in the animals supplemented with LSO as compared to the control diet and other linseed-supplemented groups. Energy loss as CH_4 which was expressed as a percentage of milk energy output was similar for the control, CLS, and ELS diets (28.7% of milk energy on average), but was less for the LSO diet (15.7% of milk energy). The inhibition of the ruminant methanogenesis may increase with the theoretical availability or release pattern of linseed FA (LSO > ELS > CLS) in the rumen (Martin *et al.*, 2008). The reduced fibre digestibility explained the decrease in CH_4 production that occurred when diets were supplemented with CLS and ELS. Similar results were confirmed by Chung *et al.* (2011) that the lower enteric CH_4 production in the non-lactating cows supplemented with ground linseed (150 g/kg DM) compared to the animals fed a basal diet based on barley silage. The PUFA in free oil probably interact more rapidly with microorganisms in the rumen than FA in seeds due to evidence of a more pronounced shift of the VFA pattern toward propionate for oils than for seeds (Jouany *et al.*, 2000). This contributed to explain the lower CH_4 (g/d) in the LSO compared to other linseed forms.

Effect of Linseed Lipid on Feed Intake and Milk Yield

The effects of linseed lipid supplement on the feed intake and milk yield of dairy ruminants are inconsistent (Table 7). Milk yield was not affected by a linseed supplement (Fuentes *et al.*, 2008), and this agrees with previous studies where linseed was used in cow diets (Petit *et al.*, 2002; Ward *et al.*, 2002; Gonthier *et al.*, 2005). The higher milk yield was also published in some studies (Petit *et al.*, 2001, 2002, 2004). Moallem (2009) reported that the average daily milk production was 1.2 kg (2.7%) higher in the dairy cows supplemented with extruded linseed compared to the control diet. Petit *et al.* (2005) suggested that the high-oil source content in the diet might depress the dry matter intake; however, the addition of FA from oilseeds at approximately 30 g/kg DM has no effect on DM intake (Allen, 2000), which may explain why the milk yield of cows fed linseed lipid was not affected or even showed a positive impact in some previous studies. On another hand, Martin *et al.* (2008) concluded that lactating dairy cows fed a diet supplemented with LSO had a significantly lower DM intake and milk yield compared to the control diet, while no negative effects were found as animals were supplemented with crude linseed or extruded linseed. This decline in DM intake could not be fully explained by disturbances in rumen function, because nutrient digestibility was not affected by different linseed forms (Martin *et al.*, 2008). It is possible that the FA intake has a direct inhibitory effect on voluntary via the inhibition of ruminorecticular motility (Chilliard, 1993).

Overall, the use of linseed lipid generally increased the flow of *cis* 18:1, *trans* 18:1, CLA, 18:3n-3, and total unsaturated fatty acids at the duodenum. Ruminants fed diets supplemented with linseed lipid had greater milk n-3 fatty acids and a sum of unsaturated fatty acids, and lower ruminal methane production.However, linseed lipid may have some negative effects on nutrient digestibility, microbial protein synthesis, and milk yield as supplementation at inappropriate levels.

Conclusions

The conclusion is that linseed lipid has a high potential to increase milk fatty acid quality and mitigate methane emission in ruminant feeding. Further researches should consider the negative effects of linseed lipid supplement on feed intake, nutrient digestibility, and milk yield in dairy animals.

Acknowledgements

The authors would like to express sincere thanks to the Research Fund of Suranaree University of Technology, Thailand for financial support in this study.

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