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Feeding oil *versus* calcium salt of n-6 and n-3 fatty acid on feed intake, digestibility, enteric methane emission and blood metabolic profile in cattle

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Abstract

A study was conducted to know effects of dietary oils and calcium salts (Ca-salts) of n-6 and n-3 fatty acid (FA) on feed intake, digestibility, enteric methane and blood metabolic profiles in bull. Four rumen cannulated bulls were used in 4x4 Latin Square Designs and supplied with Napier silage and concentrate mixture. Four dietary treatments were sunflower oil (SFO; n-6), linseed oil (LSO;n-3), Ca-salt of SFO (Ca-SFO; n-6) and Ca-salt of LSO (Ca-LSO; n-3). Oils and salts were mixed with concentrate part of the ration at 3% (w/w). Data were analyzed for ANOVA in 2x2 Factorial arrangements. It was found that, intake of DM ($p<0.05$), OM ($P<0.01$) and ADF ($p<0.05$) were reduced by feeding Ca-salts compared to oil sources. On the other hand, intake of CP ($p<0.01$) and NDF ($p<0.05$) was reduced by Ca-salts of n-3 FA only, but not of n-6 FA. The EE intake was affected by both FAs and their sources ($p<0.01$). Digestibility of DM was found higher ($p<0.05$) in n-3 FA and further Ca-salts reduced ($p<0.05$) DM digestibility. The CP ($p<0.01$) and ADF ($p<0.01$) digestibility was reduced by Ca-salts of either FA, while NDF digestibility was increased ($p<0.01$) by Ca-salt only in n-3 FA but not in n-6 FA. Concentrations of plasma glucose, urea nitrogen (BUN), cortisol and IgF-1 were affected neither by FA types nor by its sources ($p>0.05$). Concentrations of total cholesterol, IgG and insulin were decreased ($p<0.01$) by Ca-salt of FA, while triglyceride was decreased ($p<0.05$) by n-6 FA. The HDL was found to increase by n-3 FA ($p<0.01$) as well as Ca-salts ($p<0.01$) of both FA, but LDL was decreased by n-3 FA ($p<0.01$) as well as Ca-salts ($p<0.01$) of both FA. Methane production (% of total gas) were significantly reduced ($p<0.01$) by n-3 FA at 0 and 4 h after feeding, but not at 2h, while Ca-salts irrespective of FA reduced ($p<0.05$) methane in all different time periods. Oil sources compared to Ca-salts. In conclusion, Ca-salts of FAs hampered nutrient digestibility but helped to improve lipid profiles in plasma by reducing total cholesterol and LDL, but increasing HDL.

Keywords: Fatty acid, n-6 and n-3, methane emission, blood metabolic profile.

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Introduction

Many research experiments have been shown, dietary fat play significant role for boosting metabolizable energy (ME) concentration of dairy cattle and consequently improving dairy production (Beauchemin *et al.*, 2009; Palmquist *et al.*, 1986). However, over a decade, fat supplementing strategies being emphasized

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the alteration of specific FA compositions in tissues or products those have one or more biologically important functions. The polyunsaturated FA (PUFA), especially the n-6 and n-3 FA were of major concern (Amanullah, 2015). Dairy cows in our country, especially high yielding lactating animals on the other hand, are frequently face calcium shortage in their production time. Therefore, a source of calcium, having numerous biological benefits could be a smart strategy for increasing milk production in terms quality and quantity. Though, fat supplementation also increases energy density of the diet, but high dietary fat can lead to a reduction in fibre digestion in the rumen and a decline in milk fat percentage, depending on the amount and type of fat fed (Palmquist *et al.*, 1986). Again, FAs undergo an extensive degradation process in the rumen called bio-hydrogenation (Carriquiry *et al.*, 2008) in which, rumen microbes convert Polyunsaturated FA (PUFA) to several isomers and finally to saturated FA (Beam *et al.*, 2000). In order to counter these undesirable effects, dietary supplementation of fat as a Ca-salt of long chain FAs is a good alternative (Sarker *et al.,* 2012; Chalupa *et al.*, 1984). Saturated and unsaturated long chain FAs have less effect on rumen fermentation when supplemented as calcium salt than as free FAs (Chalupa *et al.*, 1985). The calcium salts of FA are insoluble at ruminal pH over 6 that can protect FA from microbial fermentation (Voigt *et al.*, 2006). Fat supplementation to some extent has been shown to be a good option of reducing enteric methane emission (Hristov *et al.*, 2013; Martin *et al.*, 2010).

Earlier, many studies were conducted taking different oil sources and Ca-salts of FA into consideration for animal feeding; however, very few have considered Ca-salts produced from the same oil. Therefore, in this srudy, n-6 and n-3 FA rich oils (sunflower oil and Linseed oil) and Ca-salts produced from same oils has been considered to know whether the Ca-salts of n-6 and n-3 FA could be better option rather feeding their oil sources directly for improving nutrient intake, digestibility, methane emission and blood metabolic profile of bulls.

Materials and Methods

The experiment was conducted at Research Farm in Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.

Animals and experimental design

Four Red Chittagong Bulls (Avg. BW 366 kg) fitted with ruminal cannula; were housed individually in digestion crates. The Bulls were randomly assigned in 4 x 4 Latin squire design (LSD) i.e. four treatment diets were rotated to four animals in four different time periods. The experiment consisted of four 15 days periods, which included 10 days for adaptation and 5 days for sample collection.

Experimental diets and feeding

During the experimental period animals were supplied basal diet with *ad libitum* Napier (pakchong) silage and concentrate. The ingredients, chemical and FA compositions of concentrate mixture was given in Table 1. Daily diet allowance was divided into two halves and supplied two times in a day; at 07.30am and 2.30pm. Clean and fresh drinking water was supplied *ad libitum* throughout the day. The concentrate mixture was supplied at a rate of 2.2% of the body weight on DM basis. Four dietary treatments were sunflower oil (SFO; oil source of n-6

Table 1. Ingredients, chemicals and fatty acid composition of experimental diets

Ingredients (%)	Silage	Experimental Diets			
		SFO	Ca-SFO	LSO	Ca-LSO
Broken wheat		37.00	37.00	37.00	37.00
Grass peabran		25.00	25.00	25.00	25.00
Wheat bran		20.00	20.00	20.00	20.00
Oil cake		12.00	12.00	12.00	12.00
Dicalcium phosphate		2.50	2.50	2.50	2.50
Common salt		0.50	0.50	0.50	0.50
SFO		3.00	-	-	-
LSO		-	-	3.00	-
Ca-SFO		-	3.00	-	-
Ca-LSO		-	-	-	3.00
Chemical composition (% DM basis)					
Dry matter (DM)	22.48	88.49	87.73	88.62	87.84
Organic matter (OM)	92.12	90.29	89.36	89.90	89.38
Crude protien (CP)	6.08	12.29	11.76	13.70	11.18
Ether extract (EE)	2.74	4.25	3.66	5.47	4.73
Acid detergent fibre (ADF)	54.95	19.56	18.76	20.25	19.64
Neutral detergent fibre (NDF)	85.70	57.13	63.88	62.59	65.26
Ash	7.88	9.71	10.64	10.10	10.62
Fatty acid composition (% of total FA)					
Myristic acid (C14:0)	1.46	0.88	0.40	0.82	0.36
Palmitic acid (C16:0)	25.00	18.11	15.14	19.06	16.54
Stearic acid (C18:0	7.87	4.94	2.28	6.91	2.27
Oleic acid (C18:1)	34.07	14.33	21.01	16.15	26.34
Linoleic acid (C18:2n-6), n-6	24.14	60.23	60.16	7.02	15.04
Linolenic acid (C18:3n-3), n-3	7.05	trace	trace	47.66	37.98
Arachidic acid (C20:0)	0.24	1.07	0.87	2.09	1.26

FA), linseed oil (LSO; oil source of n-3 FA), Ca-salt of SFO (Ca-SFO; Ca-salt of n-6 FA) and Ca-salt of LSO (Ca-LSO; Ca-salt of n-3 FA).

Data collection and sampling of feeds, orts and feces

Feed refusal (orts) was weighed every day and intake was calculated by subtracting refusal from the supplied amount. Representative samples of silage and concentrate mixer were collected at the beginning and at the completion of each experimental period. Later the samples were composited by treatments, oven dried, ground in Willy mill followed by sieving through 1.0 mm screen and preserved for chemical analysis. During collection period, every day before morning feeding, daily amount of orts were weighted, collected and preserved. After each period, 5 days ort samples were composited by animals and preserved for chemical analysis. Total feces voided daily were weighed and 10% of representative sample was collected and preserved in freezer. Feces DM was

determined daily to find the average DM in feces. After each collection period, samples were composited by animals, dried in oven at 65°C for 48 hours (LABTRON, LDO-C13, Labtron Equipment Ltd, UK), ground in Willey mill and sieved through 1.0 mm screen for chemical analysis.

Blood plasma collection

On the 5th day of each collection period, 15 ml blood sample was collected from jugular vein into vacuum tube containing additives (K2EDTA vacuum tube, REF K8050ED). Blood sample was collected at 3 hours after morning feeding. Immediately after blood collection, vacuum tubes were placed in ice box and carried to laboratory to obtain plasma by centrifuging at 3000 RPM for 10 min at 4°C (LABOGENE, Floor-type centrifuge, DK-3450 Lynge, Denmark). After centrifuging, the plasma was collected and stored at -70°C freezer (INTERTEK, U410-86, New Brunswick Scientific, England) until analyzed.

Collection of rumen fluid

Rumen fluid sample was collected on 4th day of collection period from each animal before (0 hour) and after 3 and 6 hours of morning feeding. The sample was collected with a device made by stomach tube fitted with suction syringe. After collecting the fluid sample, it was strained through four layers of cheese cloth. Immediately after collection, pH of rumen fluid was measured with pH meter (sensIONTM+ PH3) and preserved at -70°C for further analysis.

Enteric methane emission analysis

Enteric methane emission was measured in two consecutive days during each collection period directly from rumen by using portable gas analyzer (Geotech 5000). The input pipe of gas analyzer was fitted with a one-way gas collection bag that was connected to the rumen cannulae with a silicon pipe. The silicon pipe, fitted with rumen cannulae by a rubber catheter, was collected enteric gases into the one way gas collection bag voided by animal and then gas analyzer inspired the collected gases through its input pipe. The gas emission was measured in percentages of total gas inspired by the analyzer for 30 seconds.

Chemical analysis

The chemical composition of feed, orts, silage and feces samples were analyzed according to the methods described in AOAC (1995). The Dry matter (DM) content was determined by convection the samples in drying oven (LABTRON, LDO-C13, Labtrone Equipment ltd., UK) at 105°C for 24 hours. Ash was determined by incinerating the samples at 550°C for 3 hours in electric furnace (LHMF-10°C, LABNICS Equipment, USA). Crude protein (CP) content was determined by quantifying N in N analyzer followed by multiplying N content with 6.25. Ether extract (EE) content was analyzed by extracting the samples with ether in Soxhlet apparatus (SOXTEST, SX-6MP, Raypa, Spain) for 2 hours. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest *et al.* (1991). Blood metabolic profiles from plasma concentration of glucose, urea nitrogen, total cholesterol, HDL cholesterol, LDL cholesterol triglycerides, serum cortisol, immunoglobulin G (IgG), insulin like growth factor-1 (IGF-1) and serum insulin were determined by using different immunological and biochemical assay.

Statistical analysis

Data were subjected to analyzed for Analysis of Variance (ANOVA) in 2x2 factorial arrangement using repeated measures in General Linear Model (GLM) in SPSS 20, where, Factor 1 was FA (n-6 *vs* n-3) and factor 2 was sources of FA (oil *vs* Ca-salt). Mean differences were tested for significance by using Tukey's test at $p<0.05$.

Results and Discussion

Chemical composition of experimental diets

The DM content of Napier Pakchong silage was 22.48 %, where DM contents of concentrates were within 87.73 to 88.62% (Table 1). The CP content in silage was 6.08%, while concentrates CP were within 11.18 to 13.70%. The EE content was varied from 3.66 to 5.47% in treatment diets with oil and salt sources of n-3 and -6 FA, where the silage contain 2.74%. In the formulated four diets the C18:2n-6 (n-6) FA concentration were 60.23, 60.16,7.02 and 15.04% of total FA content in SFO, Ca-SFO, LSO and Ca-LSO, respectively (Table 1). On the other hand, C18:3n-3 (n-3) FA concentrations were 47.66 and 37.98% in LSO and Ca-LSO, respectively, while trace amount were found in SFO and Ca-SFO. In the silage, n-6 and n-3 FA content were 24.14 and 7.05%, respectively.

Nutrient intake and digestibility

Effects of dietary sources of n-6 and n-3 FA on nutrient intake and digestibility in bull were illustrated in Table 2. Table showed that, intake of DM ($p<0.05$), OM ($p<0.01$) and ADF ($p<0.05$) were reduced by feeding Ca-salts compared to respective oil sources. On the other hand, intake of CP ($p<0.01$) and NDF ($p<0.05$) was reduced by Ca-salts of n-3 FA only, but not of n-6 FA. Interaction effect ($p<0.01$) between FAs (n-6 vs n-3) and their sources (oil vs Ca-salt) was observed in CP and NDF intake. The EE intake was affected by both FAs and their sources ($p<0.01$). Digestibility of DM was found higher ($p<0.05$) in n-3 FA and further Ca-salts reduced ($p<0.05$) DM digestibility. The CP ($p<0.01$) and ADF ($p<0.01$) digestibility was reduced by Ca-salts of either FA, while NDF digestibility was increased ($p<0.01$) by Ca-salt of n-3 FA only, but not by n-6 FA. The reduced intake of DM and other nutrients in Ca-salt fed animals may be attributed to the reduced palatability of diets due to soapy odor of Ca-salts of FA as described by Palmquist *et al.* (1989). Lower DM intake in Ca-salt treatments can also be ascribed to the fact that the added inert fat was likely to have remained largely unavailable in the rumen because of its low solubility and high melting point (Canale *et al.* 1990). Thereby not impairing rumen fibre digestibility and avoiding an increase in gut fill that can limit DM intake. The reduced ADF and NDF digestibility in Ca-salt fed group may be due to limiting of fibre degrading microbial activity in the rumen in those animals. Klusmayer *et al.*, (1991) suggested that Ca-salts are partially protected from rumen microorganisms which may reduce fiber digestion in the rumen due to reduced of activity of protozoa and cellulolytic bacteria. Perhaps Ca-salt of SFO in this experiment was not toxic to fibrolytic organisms at such level and thereby, NDF digestibility was not affected in Ca-salt of SFO fed group. The EE intake and DM, OM and EE digestibility were significantly ($P<0.01$) increased in n-3 FA compared to n-6 FA diet irrespective of

sources. In agreement with the present findings, many previous studies also suggested that intake and digestibility of nutrient was affected by dietary supplementation of n-6 or n-3 FA sources through oil or Ca-salt of FA. Martin *et al.*, (2008) observed a strong decrease in DM intake and digestibility when linseed oil was added at 5.75% of the diet. In contrary with the above findings, many previous studies had showed intake and digestibility were not affected by the supplementation of oil or salt of n-6 and n-3 FA sources at different levels and forms in dairy or beef cattle (Reis *et al*., 2012; Benchaar *et al*., 2012).

Table 2. The dietary effect of oil and Ca-salt of n-6 and n-3FA sources nutrient intake and digestibility

Parameters	Treatments				SEM	*P*-value		
	n-6		n-3			FA	Source (S)	FA x S
	Oil	Ca-salt	Oil	Ca-salt				
Intake, Kg/d								
DM	6.35	6.13	6.52	6.16	0.119	0.416	<0.05	0.553
OM	5.71	5.50	5.95	5.40	0.121	0.573	<0.01	0.184
CP	0.54	0.51	0.57	0.47	0.008	0.446	<0.01	<0.01
EE	0.21	0.19	0.25	0.21	0.005	<0.01	<0.01	0.096
ADF	2.50	2.47	2.68	2.46	0.044	0.070	<0.05	0.057
NDF	4.72	4.71	5.01	4.46	0.098	0.841	<0.05	<0.05
Digestibility (%)								
DM	56.09	55.10	63.33	56.25	1.535	<0.05	<0.05	0.071
OM	60.13	60.09	65.98	61.35	1.116	<0.01	<0.05	0.062
CP	62.84	59.83	63.47	56.51	1.131	0.257	<0.01	0.106
EE	46.02	42.89	56.70	49.42	1.662	<0.01	<0.01	0.235
ADF	37.98	34.21	40.56	34.47	1.181	0.251	<0.01	0.345
NDF	54.99	54.72	59.39	60.35	1.260	0.568	<0.01	<0.05

DMI, Dry matter in take; DM, Dry matter; OM, Organic matter; CP, Crud protein; EE, Ether extract; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; FA, n-6 or n-3 fatty acid and Source, oil or salt of n-3 and n-6 FA

Blood metabolic profiles

Effects of dietary oil and Ca-salt of n-6 and n-3 FA sources on plasma metabolic profiles are described in Table 3. The plasma glucose, blood urea nitrogen (BUN), cortisol and IgF-1 were neither affected (p>0.05) by FA (n-6 or n-3), nor by FA sources (oil or Ca-salt). These result was evidenced from some previous observations (Moallem, 2009; Selberg *et al.* 2004), suggested that plasma glucose concentrations in ruminants does not affected by supplementing n-6 or n-3 FA sources. However, total cholesterol, LDL and IgG were reduced (p<0.01) by both the Ca-Salt of n-6 and n-3 FA. On contrary, HDL and insulin content was increased (p<0.01) by the Ca-salts irrespective of n-6 or n-3 FA. In agreement with the present findings, many previous studies also found consistent increase of total cholesterol concentrations by dietary supplementation of n-6 or n-3 FA through oil sources (Bhatt *et al*., 2011; Bindel *et al.*, 2000). Studies also suggested that the higher formation of insulin reduce total cholesterol content. The HDL and triglyceride concentration was found higher (p<0.05) in n-3 FA irrespective of sources, while LDL was reduced (p<0.01) by the same. In agreement with the present findings, many previous studies suggested that,

Table 3. Effect of dietary oil and Ca-salt of n-6 and n-3FA sources on plasma metabolic profiles

Parameters studied	Treatments				SEM	*P*-value		
	n-6 FA		n-3 FA			FA	Source	FAxSource
	Oil	Ca-salt	Oil	Ca-salt				
Glucose(mmol/L)	3.90	3.80	3.60	3.58	0.157	0.121	0.698	0.816
BUN, mg/dl	25.38	29.03	26.63	28.50	1.317	0.788	0.058	0.513
Cholesterol, mg/dl	126.95	122.67	124.89	121.04	0.938	0.072	<0.01	0.822
HDL, mg/dl	69.06	70.36	73.57	78.40	1.007	<0.01	<0.01	0.105
LDL, mg/dl	55.03	51.51	48.71	42.29	1.276	<0.01	<0.01	0.279
Triglyceride, mg/dl	9.54	9.49	10.05	10.76	0.299	<0.05	0.284	0.227
Cortisol, µg/dl	1.08	1.08	1.05	1.09	0.095	0.938	0.816	0.857
IgG, ng/dl	13.93	11.54	14.92	10.95	0.367	0.600	<0.01	0.053
IgF-1, g/L	1.03	1.15	1.01	1.08	1.276	0.739	0.479	0.856
Insulin, µIU/ml	1.55	2.64	1.46	2.49	0.938	0.072	<0.01	0.822

FA, n-6 or n-3 fatty acid and Source, oil or salt of n-3 and n-6 FA; BUN, Blood urea nitrogen; HDL, High density lipoprotein; LDL, Low density lipoprotein; IgG, Immunoglobulin G; SEM, Standard Error of Mean

supplementation with n-6 and n-3 FA increased cholesterol availability (Grummer and Carroll, 1991; Son *et al*., 1996), and Ca-salts of n-6 and n-3 may also increase circulating concentrations of insulin (Williams and Stanko, 2000).

Rumen pH and enteric methane emission

Effects of dietary oil and Ca-salt of n-6 and n-3 FA sources on ruminal pH and methane (% of total gas) emission were described in Table 4. It was observed that, rumen pH was unaffected ($p>0.05$) by both types of FA (n-6 vsn-3) and FA sources (oil vs Ca-salts). On the other hand, methane emission was significantly ($p<0.05$) reduced by oil sources compared to Ca-salts at all three time periods studied (0, 3 and 6 h after feeding; $p<0.01$, $p<0.01$ and $p<0.05$, respectively) irrespective of types of FA (n-6 and n-3). On average, n-3 FA reduced ($p<0.01$) methane emission more efficiently than n-6 FA at 0 h and 6 h after feeding, however, this effect was absent at 3 h ($p>0.05$) after feeding. Reduced methane emission by n-6 and n-3 FA rich oil in rumen is associated with toxic effects on

Table 4. Effect of dietary oils and Ca-salts of n-6 and n-3 FA sources on rumen pH and enteric methane emission

	Treatments				SEM	P-value		
	n-6		n-3			FA	source	FA*Source
	oil	salt	oil	salt				
pH								
0 h	7.40	7.37	7.34	7.29	0.098	0.32	0.53	0.89
3 h	6.93	6.90	6.90	6.88	0.152	0.81	0.78	0.61
6 h	6.68	6.73	6.43	6.62	0.236	0.30	0.48	0.68
Methane emission, (%)								
0 h	7.77	9.99	3.09	5.46	0.657	<0.01	<0.01	0.914
3 h	12.92	17.82	12.64	15.70	0.869	0.194	<0.01	0.311
6 h	10.93	13.08	7.90	8.78	0.683	<0.01	<0.05	0.371

FA, n-6 or n-3 fatty acid and Source, oil or salt of n-3 and n-6 FA.

methanogenic bacterial population (Mousasavi *et al.*, 2007; Dong *et al*., 1997). Hook *et al.*, (2010) showed that increased amount of oil in ruminant diets caused a decreased methanogenesis as a result of reduction in ciliates

Conclusion

Results suggested that Ca-salt of n-6 and n-3 FA has reduced DM intake and nutrient digestibility, while reduced total cholesterol and LDL, but increased HDL concentration in plasma of cattle. Results also suggested, oils compared to salt of n-3 and n-6 FA reduced methane emission efficiently, while n-3 FA source reduced more effectively compared to n-6 FA.

References

Amanullah, S. M. 2015. Effect of dietary n-6 and n-3 fatty acid sources on rumen fermentation characteristics and microbial diversity in ruminant. A thesis for the degree of Doctor of Philosophy. The Graduate School of Gyeongsang National University, Division of Applied Science.

AOAC. 1995. Official method of analysis, 15th edition. Association of Official Analytical Chemist. Washington DC.

Beam, T. M., Jenkins, T. C., Moate, P. J., Kohn, R. A. and Palmquist, D. L. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J. Dairy Sci. 83: 2564-73.

Beauchemin, K. A., McGinn, S. M., Benchaar, C. and Holthausen, L. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets; effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92: 2118–2127.

Benchaar, C., Romero-Pérez, G. A., Chouinard, P. Y., Hassanat, F., Eugene, M. Petit, H. V. and Côrte. C. 2012. Supplementation of increasing amounts of linseed oil to dairy cows fed total mixed rations: Effects on digestion, ruminal fermentation characteristics, protozoal populations, and milk fatty acid composition. J. Dairy Sci. 95:4578-4590.

Bhatt, R. S., Soren, N. M., Tripathi, M. K. and Karim, S. A. 2011. Effects of diffenren levela os concobut oil supplementation on performances, digestibility, rumen fermentation carcass traits of Malpura lambs. Anim. Feed Sci. Technol. 164: 29-37.

Bindel, D. J., Drouillard, J. S., Titgemeyer, E. C., Wessels, R. H. and Loest, C. A. 2000. Effects of ruminally protected choline and dietary fat on performance and blood metabolites of finishing heifers. J. Anim. Sci. 78: 2497-2503.

Canale, C. J., Burgers, P. L., Muller, L. D. and Varga, G. A. 1990. Calcium salts of fatty acids in diets that differ in neutral detergent fibre (NDF): Effect on lactation performance and nutrients digestibility. J. Dairy Sci. 73:1031-1038.

Carriquairy, M., Weber, W.L., Baumgard, L. H. and Crooker, B.A. 2008. In vitro bio-hydrogenation of four dietary fats. Fed Sci. Technol.141:339-355

Chalupa, W., B. Veechiarelli, D. Sklan and Kronfeld, D. S. 1985. Response of rumen microorganisms and lactating cows tocalcium salts of long chain fatty acids. J. Dairy Sci. 68(1):110.

Chalupa, W., Rickabaugh B., Kronfeld D. S. and Sklan D. 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. J. Dairy Sci. 67:1439-1444.

Dong, Y., Bae, H. D., McAllister, T. A., Mathison, G. W. and Cheng, K.J. 1997. Lipid-included depression of methane production and digestibility in the artificial rumen system (RUSITEC). Canadian Journal of Animal Science. 77: 269–278.

Grummer, R. R., and Carroll, D. J. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69:3838–3852.

Hook S.E., Wright A.D.G. and McBride B.W. 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, 2010, Article No. 945785.

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H. P. S., Adesogan, A. T., Yang, W., Lee, W., Gerber, P. J., Henderson, B. and Tricarico, J. M. 2013. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91 (11): 5045-5069.

Klusmeyer, T. H., Lynch, G. L., Clark, J. H. and Nelson, D. R. 1991. Effects of Calcium salts of fatty acids and protein source on ruminal fermentation and nutrient flow to duodenum of dairy cows. J. Dairy Sci. 74:2206-2219.

Martin, C., J. Rouel, J. P. Jouany, M. Doreau, and Y. Chilliard. 2008. Methane output and diet digestibility in response to feeding dairy cow's crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86:2642–2650.

Martin, C., Morgavi, D. P., and Doreau, M. 2010. Methane mitigation in ruminants: From microbe to the farm scale Anim. Feed Sci. Techno l4:351–365.

Moallem, U. 2009. The effects of extruded flaxseed supplementation to high-yielding dairy cows on milk production and milk fatty acid composition. Anim. Feed Sci. Technol. 152:232-242.

Moussavi, A. R. H., Gilbert, R. O., Overton, T. R., Bauman, D. E. and Butler, W. R. 2007. Effects of feeding fish meal and n-3 Fatty acids on milk yield and metabolic responses in rarly lactation diary cows. J. Dairy Sci. 90: 145-154.

Palmquist, D. L., Jenkins, T. and Joyner, A. 1986. Effect of dietary fat and calcium source on insoluble soap formation in the rumen. J. Dairy Sci. 69 (4), 1020–1025.

Palmquist, D. L., Leorch, S. C., Grum, D. E., Fluharty, F. L., Hughes, S. and Sweency, T. F. 1989. Acceptability of diets after abrupt introduction of Megalac. J. Dairy Sci. 72 (Suppl. 1): 443 (Abstr.).

Reis, M., Cooke, R. and Ranches, J. 2012. Effects of calcium salts of polyunsaturated fatty acids on productive and reproductive parameters of lactating Holstein cows. J. Dairy Sci. 95 (12), 7039–7050.

Sarker, N. R. Huque, K.S. Islam, H. and Das, N. 2012. Effect of feeding ca-salts of fatty acids on fatty acids composition in milk. Bang. J. Liv. Res., 19(1-2): 10-17.

Selberg, K. T., Lowe, A. C., Staples, C. R., Luchini, N. D. and Badinga, L. 2004. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids. J. Dairy Sci. 87: 158–168.

Son, J., Grant, R. J. and Larson, L. L. 1996. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. J. Dairy Sci. 79:822–830.

Voigt, J., Khula, S., Derno, M. and Hagemeister, H. 2006. Digestibility of rumen protected fat in cattle. Slovak J. Anim. Ssci. 39: 16-19.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci.74: 3568-3597.

Williams, G. L., and Stanko, R. L. 2000. Dietary fats as reproductive nutraceuticals in beef cattle. J. Anim. Sci. 77(E. Suppl.). 1–12.