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Effects of Different Levels of Fish Oil and Canola Oil on *in vitro* and *in vivo* Nutrient Digestibility

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Abstract: Two experiments were designated to examine the effects of different levels of fish oil and canola oil on *in vitro* and *in vivo* nutrient digestibility. Experiment 1 was performed to assess the effects of unsaturated oils (fish oil, canola oil, their combination) in three levels (2, 4 and 6% on DM basis) on *in vitro* Dry Matter (IVDMD) and Organic Matter (IVOMD) digestibility of alfalfa hay and corn silage. For both forages, oil supplementation decreased IVDMD and IVOMD significantly ($p<0.01$) and increasing oil levels significantly ($p<0.05$) decreased IVDMD and IVOMD of both forages but alfalfa hay was more susceptible to increasing oil levels than corn silage. In experiment 2, eight multiparous early lactation Holstein cows (42±12 DIM, 40±6 kg daily milk yield) were fed a total mixed ration supplemented with either 0% oil (control), 2% Fish Oil (FO), 1% Canola Oil+1% Fish Oil (COFO) or 2% canola oil according to a double 4×4 Latin square design to examine the effects of unsaturated oil on milk yield, DMI and nutrient digestibility. Each period lasted 3 weeks; experimental analyses were restricted to the last week of each period. Diets consist of 20% alfalfa, 20% corn silage and 60% concentrate. Cows were housed in tie stalls and fed the TMR two times a day to allow 5-10% orts (as-fed basis). Experimental diets had no significant ($p>0.05$) effect on milk production but DMI decreased significantly ($p<0.05$) in FO diet. Digestibility of OM and NDF were significantly ($p<0.05$) decreased in FO diet in comparison with three other diets, ADF digestibility was similar in diets. Results of the current experiments shows that combination of fish oil and canola oil in 2% of dry matter had less negative effects on nutrient digestibility of forages and total mixture rations.

Key words: Fish oil, canola oil, *in vitro*, *in vivo*, nutrient digestibility, Iran

INTRODUCTION

The energy requirement of the modern cow which has been bred for enhanced milk yield is difficult to meet during early lactation. Due to the high energy value of fat, supplemental fat is frequently added to ruminant diets to increase their energy density (Oldick and Firkins, 2000; Juchem *et al*., 2008). Although, dietary fat increase energy density of diets, its influence on nutrient supply to the animal depend on the digestibility of the fat sources and effects of supplemented fat on intake, rumen fermentation and the other diet component digestibility (Khorasani and Kennelly, 1998). Ruminal fermentation and fiber digestion often decrease by addition of fats or oils to the diet; however degree of inhibition varies with amount and type of supplemented fats (Jenkins and Jenny, 1992).

In comparison with saturated fatty acids, unsaturated fatty acids which is more presented in plant oils than animal fat sources had more inhibitory affects on ruminal ecosystem (Jenkins, 1993; Pantoja *et al*., 1994), decreased dry matter intake (Harvatine and Allen, 2006) and fiber digestibility especially in high concentrate diets (Ueda *et al*., 2003).

Consequently, the proportion of unsaturated and the ratio of unsaturated fatty acids to saturated ones appear to exert the most influence o nruminal fermentation (Oldick and Firkins, 2000).

Various *in vivo* and *in vitro* methods have been used to measure the extend of nutrient digestion in ruminant animals. The *in vitro* (Tilley and Terry, 1963) procedure has been extensively used to measure Dry Matter Digestibility (IVDMD) and Organic Matter Digestibility (IVOMD) of feed stuffs and has been the most accurate and practical laboratory method available for prediction of digestibility data for ruminants (Mabjeesh *et al*., 2000). This method has been modified and adapted for starch feeds analysis and various researchers have improved its accuracy of prediction.

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Objectives of these experiments were to determine the effects of different levels of fish oil and canola oil and their combination on *in vitro* digestion of alfalfa hay and corn silage as basal forages and to evaluate the effects of supplementing dairy diets in early lactation with fish oil, canola oil and their combination on total tract digestibility of organic matter, ADF, NDF.

MATERIALS AND METHODS

Experiment 1: Using a completely randomized design with 3×3 factorial arrangement of treatments, the effects of three levels (2, 4 and 6% of DM) of three oil sources (fish oil, canola oil and combination of fish oil and canola oil in 50:50 ratios) in an *in vitro* batch fermentation (Tilley and Terry, 1963) were studied. Treatments were assigned once to alfalfa hay and once to corn silage as forage basic which were dried in a force-air oven at 60°C and milled to pass a 2 mm screen using a wiley mill (Arthur H. Thomas, Philadelphia, PA) and were analyzed for CP, ADF and NDF.

The alfalfa hay had the following chemical composition (DM basis): 16 CP, 52.5 NDF and 35.5% ADF. The corn silage contained (DM basis): 6.9 CP, 43.2 NDF and 21.5% ADF. For each forage source, three tubes were used as control without oil supplements.

Incubation was performed using rumen fluid from two rumen fistulated sheep maintained on a diet of hay. The liquor was taken before morning feeding, strained through two layers of cheesecloth into a flask and kept under CO_2 gas at 38-39°C until used. The incubation was conducted in 90 mL^{-1} tube containing 1 g (W_1) of milled alfalfa hay or corn silage which were previously supplemented with different levels of oils, 10 mL^{-1} of rumen fluid and 40 mL^{-1} of phosphate bicarbonate buffer (McDougall, 1948). Each tube was gassed with CO_2 before sealing with rubber corks with a gas release valve. Then tubes were incubated at 38°C for 48 h with occasional shaking.

At the end of the first incubation period, 6 mL HCL (20%) and then 5 mL pepsin solution (0.5 g pepsin was solved in 100 mL HCL 0.1 N) were added gradually to each tube. The tubes were then incubated at 38°C for 48 h. Finally, the supernatant were discarded using filter papers (No. 42), the residues then were transferred to a 50 mL cruse and dried at 55°C until constant weight. The dry weight of the residue were calculated (W_2) to measure IVDMD and for IVOMD measurement, dried samples were heated at 550°C for 3-4 h.

After that samples weight was recorded (W_3). Using following formula, IVDMD and IVOMD were calculated. IVDMD = $(W_1-W_2)/W_1$ and IVOMD = $(W_2-W_3)/W_2$.

Experiment 2: Eight multiparous Holstein cows in early lactation (42±12 DIM, 40±6 kg daily milk yield) were use in a 4×4 Latin square split plot with four treatments, four periods and two cows per treatment as the main plot and the four sampling periods as the subplot. Each period lasted 21 days which included a 14 days diet adjustment period followed by a sampling period.

There were four treatments consist of diet with no supplemental oil (control) and diet supplemented with 2% Fish Oil (FO), 1% fish Oil-1% Canola Oil (FOCO) and 2% Canola Oil (CO). Diets were composed of 60% (DM) concentrate mix, 20% alfalfa hay and 20% corn silage and formulated to meet energy and protein requirements of lactating cows producing 40 kg of milk and consuming 24 kg of DM day^{-1} (Table 1).

Oils were added at a level of about 2% of dietary DM, resulting in a dietary ether extract content of 4.7%. Kilika

Table 1: Ingredient component and chemical composition of experimental diets

	Treatments[1]			
Variables	Control	FO	FOCO	CO
Ingredients (DM%)				
Alfalfa	20.00	20.00	20.00	20.00
Corn silage	20.00	20.00	20.00	20.00
Corn grain	15.00	13.00	13.00	13.00
Barely grain	15.00	15.00	15.00	15.00
Soybean meal	10.00	10.00	10.00	10.00
Canola meal	8.00	8.00	8.00	8.00
Bran	10.50	10.50	10.50	10.50
Fish oil[2]	-	2.00	1.00	-
Canola oil[3]	-	-	1.00	2.00
Limestone	0.50	0.50	0.50	0.50
Vitamin supplement	0.80	0.80	0.80	0.80
Salt	0.20	0.20	0.20	0.20
Chemical composition (DM%)				
CP	16.70	16.30	16.20	16.40
NDF	32.08	33.21	32.77	33.12
ADF	19.07	19.02	18.87	18.66
OM	93.06	92.48	92.63	92.48
NFC[4]	41.40	38.31	39.16	38.39
Ether extract	2.78	4.62	4.67	4.53
Ca	0.80	0.80	0.80	0.80
P	0.60	0.60	0.60	0.60
Mg	0.27	0.27	0.27	0.27
NE_L (Mcal kg^{-1})	1.53	1.59	1.59	1.59
Fatty acids (g/100 g fatty acids)				
C16:0	16.11	13.58	12.87	11.35
C18:0	3.02	3.73	3.35	3.29
C18:1	25.79	26.02	32.95	37.90
C18:2	32.13	22.52	24.09	26.61
C18:3	-	5.95	7.40	-
C20:0	0.60	0.70	0.78	0.60
C20:4	1.86	3.12	3.73	3.17
C20:5 EPA	1.35	2.78	2.57	1.91
C22:5	1.30	1.11	1.09	1.92
C22:6 DHA	1.75	7.98	3.17	2.08

[1]Control = diet without oil; FO = diet supplemented with fish oil (2 % DM); FOCO = Diet supplemented with 1% (DM) fish oil and 1% (DM) canola oil; and CO = diet supplemented with 2% (DM) canola oil. [2] khazar Co, Babolsar, Iran. [3]Golestan Soybean, Gorgan, Iran. [4]NFC = 100 - (NDF+CP+Ash+Ether Extract)

fish oil (Khazar Co, Babolsar, Iran) and canola oil (Golestan Soybean Co., Gorgan, Iran) were used in this experiments. The oils were added first to the part of the bran and soy bean meal to produce a premix then after well mixed were added to the other parts of the concentrate weekly. Concentrate mixtures and forage sources were mixed in a weighting and mixing unit and offered in the TMR form twice daily to allow 5-10% orts (as-fed basis). Cows were housed in free stalls with continuous access to water and were milked daily at 0500, 1200 and 2000.

Individual cow intake was recorded daily but only the Dry Matter Intake (DMI) during last 5 days of each experimental period was used for statistical analysis. Milk yield were recorded during last 7 days of each experimental period.

TMR mixture and feace were sampled on first 5 days of each sampling period and were stored in -20. At the end of each period, feed samples were mixed to get the final sample and were stored in -20 up to the end of experiment. Finally all the feed and feace samples were dried in a forced-air oven at 60°C and stored in sealed plastic containers at room temperature until analyzed.

In preparation for analyses, dried feed and feace were ground first through a 2 mm screen (Wiley; Arthur H. Thomas, Philadelphia, PA) and were analyzed for OM, ADF, NDF (Van Soest and Wine, 1967) and Acid Insoluble Ash (AIA).

Using gas chromatography method, fatty acid composition of diets and supplemental oil has been determined which are shown in Table 1 and 2, respectively. AIA content of feed and feace was used as a natural marker in ruminant to determine apparent digestibility of some nutrient using following formula:

$$\text{Apparent digestibility}(\%) = 100 - (100 \times \text{Feed AIA}(\%) / \text{Feace AIA}(\%) \times \text{Feed nutrient}(\%) / \text{Feace nutrient}(\%))$$

Table 2: Fatty acid composition (g/100 g of fatty acids) of supplemental oils

Fatty acids	[1]Fish oil	[2]Canola oil
C12:0	0.29	0.22
C14:0	4.42	-
C16:0	24.04	4.20
C16:1	5.99	0.25
C18:0	5.11	2.26
C18:1 (n-9)	33.64	63.75
C18:2 (n-6)	3.20	16.92
C18:3 (n-3)	1.33	8.00
C20:0	0.22	0.69
C20:5 EPA	5.07	-
C22:6 DHA	13.32	-

[1]Khazar Co, Babolsar, Iran, [2]Golestan Soybean, Gorgan, Iran

RESULTS AND DISCUSSION

Experiment 1: Results of IVDMD and IVOMD of oil supplemented alfalfa hay and corn silage are shown in Table 3 and 4, respectively. In contrast with control treatments of these two forages, including fish oil, canola oil and their combination decrease IVDMD and IVOMD of both forages significantly ($p<0.01$). IVDMD and IVOMD of alfalfa hay and corn silage decreased significantly in CO treatments in comparison with two other oil sources ($p<0.05$) and linear increase in oil levels from 2-6% (DM) cause IVDMD and IVOMD decrease in alfalfa hay but in corn silage including 6% oil in comparison with 2% decrease IVDMD and there were no significant differences in dry matter digestibility when oil level rise from 2-4% and from 4-6%. IVOMD

Table 3: The effects of different levels of fish oil and canola oil on *in vitro* digestibility of alfalfa hay

	Parameter[1]			
Treatments	IVDMD	SE	IVOMD	SEM[2]
Control	71.400	1.06	69.980	0.91
Oil[3]				
FO	69.100[a]	0.83	66.400[a]	0.81
CO	66.200[b]	-	63.200[b]	-
FOCO	70.000[a]	-	68.400[a]	-
p-value	*		**	-
Level (DM%)				
2	70.800[a]	0.77	68.700[a]	0.80
4	68.400[b]	-	66.000[b]	-
6	66.000[c]	-	63.400[c]	-
p-value	0.001	-	0.001	-
p-value				
Contrast control vs. others	*		*	

[1]IVDMD = *In vitro* Dry Matter Digestibility, IVOMD = *In vitro* Organic Matter Digestibility. [2]SEM = Standard Error of Means. [3]FO = Fish Oil, CO = Canola Oil, FOCO = Fish Oil+Canola Oil (50:50). ns = not significant *p<0.05, **p<0.01. [a,b,c]Row means differ significantly

Table 4: The effects of different levels of fish oil and canola oil on in vitro digestibility of corn silage

	Parameter[1]			
Treatment	IVDMD	SEM[2]	IVOMD	SEM[2]
Control	64.30	0.79	65.40	1.40
Oil[3]				
FO	63.50[a]	0.51	61.90[a]	0.62
CO	59.10[b]	-	58.40[b]	-
FOCO	63.00[a]	-	62.50[a]	-
p-value	***		0.001	-
Level (DM%)				
2	62.90[a]	0.49	62.10[a]	0.60
4	61.90[ab]	-	60.90[ab]	-
6	60.70[b]	-	59.80[b]	-
p-value	0.04	-	0.06	-
p-value				
Contrast control vs. others	*		**	

[1]IVDMD = *In vitro* Dry Matter Digestibility, IVOMD = *In vitro* Organic Matter Digestibility. [2]SEM = Standard Error of Means. [3]FO = Fish Oil, CO = Canola Oil, FOCO = Fish Oil+Canola Oil (50:50). ns = not significant *p<0.05, **p<0.01, ***p<0.0001 [a,b,c] Row means differ significantly

of corn silage tend to be decrease when oil level increased from 2-6% ($p = 0.06$). Oil levels and sources interactions were not significant for both forage type ($p>0.05$).

The *in vitro* method of evaluating the digestibility of ruminant feeds is used worldwide. The method is easier than *in vivo* analysis and avoids the need to surgically prepared animals in different locations in the gastrointestinal tract. The TT method has also been proven more accurate than digestibility predictions based on the chemical compositions of feeds (Van Soest and Wine, 1967). Moreover, because of its similarity to *in vivo* values (Tilley and Terry, 1963), it is considered a reference method for the prediction of ruminant feed digestibility.

Fiber degradation in the rumen may be limited by several dietary factors that alter attachment by or nutrient availability to fibrolytic bacteria. Cellulolytic bacteria apparently need to be in close proximity or attached to fiber before degrading it. Among several theories discussed by Palmquist and Jenkins (1980), one states that unsaturated dietary fats may coat fiber and interfere with bacterial attachment.

As revealed in results, unsaturated oil supplementation significantly declined IVDMD and IVOMD of both forage type which is mostly related to NDF and ADF section of forages. Including unsaturated oil in diets often decrease digestibility of nutrients, spatially in forages (Avila *et al*., 2000). IVDMD of soybean oil supplemented broomgrass hay decreased significantly and similar to the results, a linear decline was noted for IVDMD of this hay as the percentage of soybean oil in diet increased from 3-6% (DM) (Whitney *et al*., 2000). However, addition of 10% (DM) of soybean oil or hydrogenated tallow did not affect *in vitro* NDF digestibility of crystalline cellulose (Firkins *et al*., 1991) which has been related to the basal diet used.

Including canola oil in comparison with fish oil or combination of fish oil and canola oil significantly decreased IVDMD and IVOMD of both forages which was probably because of higher ratio of unsaturated fatty acids to saturated fatty acids (U:S) in canola oil in comparison with fish oil. As reported previously (Avila *et al*., 2000) oil suplements with higher U:S ratio will have more adverse effect on fiber digestibility.

Alfalfa hay and corn silage had shown different responses of digestibility to oil levels. Increasing levels of oil from 2-4 and from 4-6 (DM%) significantly decreased IVDMD and IVOMD of alfalfa hay but IVDMD of corn silage was declined significantly when oil level increased from 2-6%. One of the possible reasons of these differences might be chemical composition of alfalfa hay and corn silage.

Alfalfa hay had higher percentages of ADF and NDF than corn silage which made it more sensitive to increasing oil levels. Results of this experiments show that unsaturated oils significantly decreased IVDMD and IVOMD of forages and their depends on oil type, oil levels, degree of saturation, basal diet and chemical compositions of forage.

Experiment 2: The result of milk production and DMI are shown in Table 5. Milk yield was not affected by oil supplemented diets ($p>0.05$). Supplementing diets with oils rich in Polyunsaturated Fatty Acids (PUFA) often results in a reduction in nutrient intake and milk production (Chilliard *et al*., 2001; Lock and Shingfield, 2004). However, there are several previous reports which have reported no significant effects of supplemented oil on milk yield (AbuGhazaleh and Holmes, 2007; Juchem *et al*., 2008). Donovan *et al*. (2000) also reported no significant decline in milk production until cows were consuming 3% (DM) FO. Where, by Whitock *et al*. (2002)'s study, milk production appeared to be lower numerically in cows consuming 2% FO diet in comparison with three other diets.

Including 2% (DM) fish oil in diets decreased DMI significantly ($p<0.05$) in comparison with control and other supplemented diets. Intake is regulated by the type and temporal pattern of available fuels (Allen, 2000), the interaction of available fuels and metabolic state, the potential negative effect of feeding supplemental oil on food palatability and ruminal fiber digestion. Such an effect can be expected to be most important with oils or ground seeds because of direct contact between lipids and rumen microorganisms (Bayourthe *et al*., 2000).

In the current experiment DMI was decreased just in FO diet which is probably because of the lower palatability of FO diet. Digestibility of OM and NDF were significantly decreased in FO diet in comparison with three other diets but ADF digestibility were not affected by diets (Table 5). The digestibility of structural carbohydrates often decreases in oil containing diets,

Table 5: The effect of experimental diets on DMI, milk yield and nutrient digestibility of early lactating Holstein dairy cows

	Treatments[1]					
Parameter	Control	FO	FOCO	CO	SEM[2]	p-value
Milk yield (kg day^{-1})	34.08	33.84	34.55	33.90	0.86	ns
DMI (kg day^{-1})	24.92^{a}	22.21^{b}	24.61^{a}	24.86^{a}	0.61*	
Digestibility (%)						
OM	65.58^{a}	60.62^{b}	62.98^{a}	62.33^{a}	1.05*	
NDF	57.81^{a}	50.55^{b}	53.22^{a}	53.89^{a}	2.52*	
ADF	43.63	42.11	42.42	43.72	1.05	ns

[1]Control = diet without oil; FO = diet supplemented with fish oil (2% DM); FOCO = Diet supplemented with 1% (DM) fish oil and 1% (DM) canola oil; and CO = diet supplemented with 2% (DM) canola oil.[2]SEM = Standard Error of Means. ns = not significant *$p<0.05$, **$p<0.01$, ***$p<0.0001$ ^{a,b,c}Row means differ significantly

especially when unsaturated oil sources are included in diets. However, data on the interaction between basal diet and lipid supplementation for total-tract digestibility are inconsistent and depend on different parameters such as oil level, degree of oil saturation and the basal diet (Ueda *et al*., 2003), especially forage to concentrate ration (Ikwuegbu and Sutton, 1982).

Ueda *et al*. (2003) reported higher ruminal NDF digestibility with linseed oil supplementation to the forage-rich diet whereas it decreased in concentrate-rich diet. In similar, in corn silage-based diet (Doreau and Chilliard, 1997) with around 35% concentrate ration in diet, a positive effect of n-3 fatty acids from fish oil, on ruminal fiber digestibility was reported.

However, a negative effect of 7% rapeseed oil on digestion was observed in a diet based on corn silage whereas differences were not significant with a hay-based diet (Ben Salem *et al*., 1993). High concentrate to forage ration along with high degree of unsaturation in FO diet in the current study are possible reasons for lowest NDF digestibility in FO diet. As reported, the degree of unsaturation and the number of double bounds in fatty acids of oil supplements increase negative effects of them.

Digestibility of OM in total tract was decreased significantly in FO diet which is probably related to lower NDF digestibility in FO diet. In spite of lower total tract digestibility reported in oil supplemented diets, higher levels of oil between (6-11% of DM) had no effects on total tract digestibility in lambs, sheep or in lactating dairy cows. Ueda *et al*. (2003) had also reported a significant decrease in ruminal OM digestibility with linseed treatment in high concentrate diet but total tract digestibility of OM was higher for that diet which has been referred to compensatory digestion of OM in intestine.

Faichney reported that the digestion in large intestine compensated partially for the negative effect of polyunsaturated lipids on ruminal digestion. According to lower total tract digestibility of some diet nutrients, the benefits of increased energy density associated with fat supplementation maybe lost with increasing the levels of oil in diets.

Based on results of nutrient apparent digestibility, supplementing diets with 2% (DM) fish oil would have more negative effects on some nutrient intake and digestibility than its combination with plant oils.

CONCLUSION

This study revealed that oil levels, basal diets and degree of oil saturation are the most important factors affecting nutrient digestibility of forages and total mixed rations of Holstein dairy cows. *In vitro* results revealed that canola oil had more negative effects of alfalfa hay and corn silage and digestibility of alfalfa hay was more decreased than corn silage when oil levels were increased. Total tract digestibility results illustrated that diet with 2% (DM) fish oil had lower OM and NDF digestibility of all treatments. In conclusion, combination of canola oil and fish oil had less negative effect on fiber digestibility that separately feeding them.

REFERENCES

AbuGhazaleh, A.A. and L.D. Holmes, 2007. Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. J. Dairy Sci., 90: 2897-2904.

Allen, M.S., 2000. Effects of diet on short term regulation of feed intake by lactating dairy cattle. J. Dairy Sci., 83: 1598-1624.

Avila, C.D., E.J. de Peters, H. Perez-Monti, S.J. Taylor and R.A. Zinm, 2000. Influences of saturation ratio of supplemental dietary fat on digestion and milk yield in dairy cows. J. Dairy Sci., 83: 1505-1519.

Bayourthe, C., F. Enjalbert and R. Moncoulon, 2000. Effects of different forms of canola oil fatty acids plus canola meal on milk composition and physical properties of butter. J. Dairy Sci., 83: 690-696.

Ben Salem, H., R. Krzeminski, A. Ferlay and M. Doreau, 1993. Effect of lipid supply on *in vivo* digestion in cows: Comparison of hayand corn-silage diets. Can. J. Anim. Sci., 73: 547-557.

Chilliard, Y., A. Ferlay and M. Doreau, 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially Conjugated Linoleic Acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci., 70: 31-48.

Donovan, D.C., D.J. Schingoethe, R.J. Baer, J. Ryali, A.R. Hippen and S.T. Franklin, 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci., 83: 2620-2628.

Doreau, M. and Y. Chilliard, 1997. Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. Reprod. Nutr. Dev., 37: 113-124.

Firkins, J.L., J.G.P. Bowman, W.P. Weiss and J. Naderer, 1991. Effects of protein, carbohydrate and fat sources on bacterial colonization and degradation of fiber *in vitro*. J. Dairy Sci., 74: 4273-4283.

Harvatine, K.J. and M.S. Allen, 2006. Effects of fatty acid supplements on ruminal and total tract nutrient digestion in lactating dairy cows. J. Dairy Sci., 89: 1092-1103.

Ikwuegbu, O.A. and J.D. Sutton, 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr., 48: 365-375.

Jenkins, T.C. and B.F. Jenny, 1992. Nutrient digestion fed combinations and lactation performance of dairy cows of prilled fat and canola oil. J. Dairy Sci., 75: 796-803.

Jenkins, T.C., 1993. Lipid metabolism in the rumen. J. Dairy Sci., 76: 3851-3863.

Juchem, S.O., J.E.P. Santos, R.L.A. Cerri, R.C. Chebel and K.N. Galvao *et al*., 2008. Effect of calcium salts of fish and palm oils on lactational performance of Holstein cows. Anim. Feed Sci. Technol., 140: 18-38.

Khorasani, G.R. and J.J. Kennelly, 1998. Effect of added dietary fat on performance, rumen characteristics and plasma metabolites of midlactation dairy cows. J. Dairy Sci., 81: 2459-2468.

Lock, A.L and K.J. Shingfield, 2004. Optimising Milk Composition. In: Dairying-Using Science to Meet Consumers Needs, Kebreab, E., J. Mills and D.E. Beever (Eds.). Nottingham University Press, Loughborough, UK., pp: 107-188.

Mabjeesh, S.J., M. Cohen and A. Arieli, 2000. *In vitro* methods for measuring the dry matter digestibility of ruminant feedstuffs: Comparison of methods and inoculum source. J. Dairy Sci., 83: 2289-2294.

McDougall, E.I., 1948. Studies on ruminant saliva. Biochem. J., 43: 99-109.

Oldick, B.S. and J.L. Firkins, 2000. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. J. Anim. Sci., 78: 2412-2420.

Palmquist, D.L. and T.C. Jenkins, 1980. Fat in lactation rations: Review. J. Dairy Sci., 63: l-14.

Pantoja, J., J.L. Firkins, M.L. Eastridge and B.L. Hull, 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. J. Dairy Sci., 77: 2341-2356.

Tilley, J.M.A. and R.A. Terry, 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grass. Soc., 18: 104-111.

Ueda, K., A. Ferlay, J. Chabrot, J.J. Loor, Y. Chilliard and M. Doreau, 2003. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage: Concentrate ratios. J. Dairy Sci., 86: 3999-4007.

Van Soest, P.J. and H. Wine, 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Assoc. Official Anal. Chem., So: 50-55.

Whitney, M.B., B.W. Hess, L.A.B. Balstad, J.L. Sayer, C.M. Tsopito, C.T. Talbott and D.M. Hallford, 2000. Effects of supplemental soybean oil level on *in vitro* digestion and performance of prepubertal beef heifers. J. Anim. Sci., 78: 504-514.

Whitock, L.A., D.J. Schingoethe, A.R. Hippen, K.F. Kalscheur, R.J. Baer, N. Ramaswamy and K.M. Kasperson, 2002. Fish oil and extruded soybeans fed in combination increase conjugated lionleic acids in milk of dairy cows more than when fed separately. J. Dairy Sci., 85: 234-243.