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EFFECTS OF DIFFERENT LEVELS OF FISH OIL AND CANOLA OIL ON PRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY COWS

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ABSTRACT

To examine the effects of different levels of fish and canola oils on dairy cows performance, eight early lactation cows were fed diets 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. Experimental analyses were restricted to the last week of each period. Milk production, concentration and production of milk protein, lactose and SNF were all similar between diets. The percentage and production of milk fat decreased significantly in all oil supplemented diets. DMI and CP intakes significantly decreased in FO diet, but intakes of OM, NDF and ADF did not change between diets. Fat intake and most of fatty acid (FA) intake increased significantly in supplemented diets. The blood metabolites were all similar between diets. Supplementing diets with fish oil and canola oil had no significant effects on rumen PH and N-NH_3.

Key words: Fish oil, Canola oil, Dry matter intake, Nutrient digestibility, Milk yield component.

INTRODUCTION

Supplemental fat sources are utilized in rations for dairy cows to increase the energy density of the diet or to modify milk production, milk fat content and milk fatty acids profile (Juchem *et al.*, 2008); however, 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). It is well recognized that feeding vegetable oils containing unsaturated fatty acids has the potential to inhibit ruminal fermentation, decreased dry matter intake (Harvatine and Allen, 2006b) and fiber digestibility especially in high concentrate diets (Ueda *et al.,* 2003), and also decreased milk fat percentage (Whitlock *et al.,* 2002 and Abughazaleh *et al.*, 2004). The ω-3 fatty acids also have been associated with positive effects on human health for a long time. Fish oil contains relatively high concentrations of two polyunsaturated fatty acids of the n-3 family: eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) (Doreau et al., 1999). Canola seed, one of the major oil seed produced in Iran, is one of an excellent source of dietary fat high in essential fatty acids and protein for dairy animals (Ward et al., 2002). It has been reported that milk yield of early lactating cows increased when 5% jet-sploded canola seed was included in diet, but milk protein decreased (Khorasani *et al.*, 1992). Morover, canola oil contains 62% monounsaturated fatty acid and 22% C18:2. Rumen escape of C18:1 may result in higher milk C18:1 (Ward *et al.*, 2002). As performance of Holstein dairy cows has been improved when fish oil has been combined with vegetables oils, and also the lack of research on combining canola oil and fish oil, the current study was designed to evaluate the effect of fish oil and canola oil supplemented diets on DMI, nutrient digestibility and intake, milk yield and components, and some blood metabolites and rumen parameter in high producing dairy cows in early lactation.

MATERIAL AND METHODS

Eight Holstein cows in early lactation (42±12 DIM, 40±6 kg daily milk yield) assigned to dietary treatments according to a double 4 ×4 Latin square design. Each period lasted 21 d (14-d diet

for diet adjustment, 7-d for sampling). Treatments were: Control (without oil), FO (2% DM fish oil), and FOCO (1%fish oil-1% canola oil), and CO (2% canola oil) (table 1). Oils added at a level of about 2% of dietary DM, resulting in dietary ether extract content of 4.7%. Kilika fish oil (khazar Co, Babolsar, Iran), and canola oil (Golestan Soybean Co, Gorgan, Iran) were used in this experiments. Cows were housed in free stalls with continuous access to water and were milked daily at 0500, 1200, and 2000 hrs.

Daily samples of feed and/or feace on each sampling period were taken and stored in -20°C. At the end of each period feed and/or feace samples mixed to get the final sample, stored in -20°C, and finally dried in a forced-air oven at 60°C 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 fat-(ADF) (Van Soest, 1991)-(NDF) (Van Soest et al., 1991), and (CP) (AOAC, 1990), acid-insoluble ash (AIA) (Van Keulen and Young, 1977) and fatty acid composition (table 2). AIA content of feed and feace was used as a natural marker in ruminant to determine apparent digestibility of some nutrient, using following formula: Apparent digestibility (%)= 100-[100×(feed AIA(%) / feace AIA(%))×(feed nutrient(%) / feace nutrient(%))].

Dry Matter Intake (DMI) and milk yield recorded at the sampling period. Milk samples were collected at the last two days of each period and stored at 4°C until analyzed for fat, protein, and solid not fat (SNF) (Micro Scan; FOSS Electric A/s, Denmark). Blood samples (20 ml) were taken from the coccygeal vein in the last day of each period at 2h after the morning feeding, kept on ice and centrifuged within 20 min at 3000 x g for 20 min. Aliquots of serum were stored at –20°C until analysis for glucose, insulin, triglyceride, cholesterol, and serum urea nitrogen (SUN). Ruminal fluid was collected on the last day of each experimental period 3h post feeding via stomach pump according to procedures in Dirksen and Smith (1987). After pH determination, samples were transported to the laboratory, strained through two layer cheesecloth. 10 milliliter (ml) of each sample were mixed with 10 ml HCL (0.1N), and then frozen at -20 until analyzed for ammonia ($N\text{-}NH_3$).

Data was analyzed as a replicated 4×4 Latin square using generalized linear model (PROC GLM, Inst, Inc) of SAS (1996) using the following model: $Y_{ijk}=\mu+T_i+P_j+A_k+\mathring{a}_{ijk}$, where Y_{ijk} is the dependent variable, μ is the global mean, T is the treatment effect, P is the period effect, A is the animaleffect, and $\mathring{a}_{ijk}$ is the residual error. Model effects were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Milk production and component

Milk production was not affected by diets, but fat corrected milk (FCM4%) and energy corrected milk (ECM) were decreased ($P<0.05$) for cows fed oil supplemented diets (table 3). Some researchers reported a significant decline in milk production (Chilliard *et al.,* 2001; Lock and Shingfield, 2004) and some other reported no significant effects (Palmquist and Griinari 2006; Abughazaleh et al., 2007 and Juchem *et al.,* 2008) of oil supplemented diets on milk production. One of the possible reasons in decreased milk production is the level of fish oil in diet which causes DMI to decline. Donovan et al (2000) reported no significant decline in milk production until cows were consuming 3%(DM) FO; Whereas, in Whitloch et al (2002) study, milk production appeared to be lower numerically in cows consuming 2% fish oil in diet.

Milk fat percentage and production decreased ($P<0.05$) in oil supplemented diets (Table 3). Milk fat concentration usually decreased in oil supplemented diets (Chichlowski *et al.*, 2005). Rapid availability of the oil in the diet and its potential negative effect on fiber digestibility (Abughazaleh *et al.*, 2004), incomplete biohydrogenation and production of various substrates and conjugated linoleic acid isomers including trans-10 cis-12 CLA (Baumgard *et al.*, 2001; Bauman and Griinari, 2003) are the possible reasons of lower milk fat percentage when oil containing diet were fed. Milk fat depression also reported when fish oil (Ramaswamy *et al.*, 2001; Abughazaleh *et al.*, 2004 and Shingfield *et al.*, 2006) and calcium salt of canola oil fatty acids (Chouinard *et al.*, 1997) were included in diets or 330 g of canola oil was infused into rumen (Depeters *et al.*, 2001).

Milk protein, lactose and SNF percentages and productions did not change ($P>0.05$). Milk protein concentration often decreases when fat

Table 1: Ingredient component and chemical composition of experimental diets.

Variable	Treatments[1]			
	Control	FO	FOCO	CO
Ingredients,% of DM				
Alfalfa	20	20	20	20
Corn silage	20	20	20	20
Corn grain	15	13	13	13
Barely grain	15	15	15	15
Soybean meal	10	10	10	10
Canola meal	8	8	8	8
Bran	10.5	10.5	10.5	10.5
Fish oil[2]	-	2	1	-
Canola oil[3]	-	-	1	2
Limestone	0.5	0.5	0.5	0.5
Vitamin supplement	0.8	0.8	0.8	0.8
Salt	0.2	0.2	0.2	0.2
Chemical composition ,% of DM				
CP	16.7	16.3	16.2	16.4
NDF	32.08	33.21	32.77	33.12
ADF	19.07	19.02	18.87	18.66
OM	92.06	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	0.8	0.8	0.8	0.8
P	0.6	0.6	0.6	0.6
Mg	0.27	0.27	0.27	0.27
NE_L, Mcal/Kg	1.53	1.61	1.59	1.60

[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)

sources are included in dairy diets (Shingfield *et al.*, 2006; Juchem *et al.,* 2008). A direct effect of fish oil fatty acids on protein synthesis or amino acid uptake in the mammary gland reported, is because of negative effects of dietary fat on somatotropin release (Petit *et al.*, 2002). Energy intake (Lock and Shingfield, 2004), reduced availability of glucose (Smith *et al.*, 1978), decreased rumen concentration of propionate, and low plasma insulin concentration have all been implicated as possible causative factors for milk protein decrement with dietary fat supplementation. Davis (1990) speculated that the degree of unsaturation of long-chain fatty acids that reached the small intestine might be associated with decreased milk protein percentage. However, formaldehyde-protected canola seed (Delbecchi *et al.*, 2001) and canola oil supplemented diets (Khorasani *et al.*, 1998) increased milk protein concentration significantly.

Blood Metabolites

Concentration of blood metabolite was similar between diets (P>0.05; Table 3). Similarly, previous research reported no effects of fat supplementation in glucose concentration and insulin secretion (Delbechi *et al.,* 2001). In contrast, Heravi Moussavi et al (2007) reported that cows fed 5% fish meal and 2.3% calcium salt of fish oil fatty acid had the greatest glucose concentrations. When unsaturated FA in a calcium salt form replaced hydrogenated prilled FAs, plasma insulin concentration decreased by 27% (Harvatine and Allen, 2005), but with an increase in unsaturated long-chain FAs in diet, insulin secretion in rat pancreases increased lineary (Opara *et al.*, 1994). Insulin secretion is also affected by ruminal propionate production which is the most important substrate for gluconeogenesis (Drackley *et al.*, 2001) and plays a predominant stimulatory role in insulin secretion. Blood concentration of triglyceride

Table 2: Fatty acid composition (g/100 g of fatty acids) in experimental diets.

Fatty Acids	Treatments[1]			
	Control	FO	FOCo	CO
12:0	0.22	0.12	0.11	0.11
14:0	0.43	1.4	0.85	0.38
14:1	-	0.16	0.07	0.01
16:0	16.11	13.58	12.87	11.35
16:1	0.19	0.2	0.1	0.07
18:0	3.02	3.73	3.35	3.29
18:1 *trans*	0.05	0.04	0.06	0.04
18:1 *cis-9*	23.17	23.62	30.17	34.89
18:1 *cis-11*	2.57	2.36	2.72	2.97
18:2 *trans-9, trans-12*	0.06	0.02	0.06	0.05
18:2 *cis-9, cis-12*	32.7	22.5	24.03	26.56
18:2 *trans-10, cis-12*	ND	ND	0.04	0.07
18:2 *trans-9, trans-11*	ND	ND	0.06	0.01
18:3	6.11	5.95	7.14	6.65
20:0	0.6	0.7	0.78	0.6
20:4	1.86	3.12	3.73	3.17
20:5 *EPA*	0.08	2.78	1.57	0.14
22:5	1.3	1.11	1.09	1.92
22:6 *DHA*	0.05	3.09	1.12	0.12

[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.

(P=0.67) and cholesterol (P= 0.40) were both similar between diets. The concentration of triglycerides in serum increases when cows receive increasing levels of dietary FAs from canola seed (LaCount *et al.*, 1994). In their study it has been proposed an increase in blood triglyceride concentration relate to the capability of the epithelium of the small intestine in absorbing the dietary FAs postruminally and incorporating them into triglyceride lipoproteins. Including 14%(DM) ground canola seed had no effects on serum glucose and free FAs, but serum triglyceride concentration increased significantly in diet with ground canola seed (Chichlowski *et al.*, 2005). It was demonstrated (Drackley *et al.*, 1992; Choi *et al.*, 1996) a positive relationship between serum cholesterol concentration and dietary fat. Nestel et al (1978) proposed that an increase in dietary fat stimulates intestinal cholesterol synthesis to meet the increased demand for absorption and transport of fat.

Nutrient intake and digestibility

The effects of experimental diets on nutrient intake and digestibility are presented in Table 4. FA intake of experimental diets is shown in table 5. Cows fed FO diet had the lowest DMI (p<o.o5), which is a typical response to fish oil supplementation (Donovan *et al.*, 2000; Whitlock et al., 2002), especially when the levels of fish oil increase in diet (Keady and Mayne, 1999; Palmquist and Griinari, 2006). CP intake increased in FO diet, but intake of OM, NDF and ADF were all similar between diets (P>0.05). Fat intake increased (P<0.05) in oil containing diets. Intake is regulated by the type and temporal pattern of available fuels, the interaction of available fuels and metabolic state, the potential negative effect of feeding supplemental oil on food palatability and ruminal fiber digestion (Allen, 2000). 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). Increasing unsaturated FAs (Harvantine and Allen, 2006b), Abomasal infusion of unsaturated fat (Benson and Reynolds, 2001) and unsaturated FA with a lower C16:C18 FAs ratio (Drackley *et al.*, 1992 and Christensen *et al.*, 1994) decreased DM and energy intake. In the current experiment DMI decreased just in FO diet, which is probably because of the lower palatability and higher proportion of highly unsaturated FAs of FO diet. CP intake was lower in FO diet which relates to lower DMI in this diet. Supplemental oils had no negative effects on OM intake which is probably resulted from

high level of fat in oil supplementing diets. Intakes of FAs were significantly different between diets (Table 5). The EPA intake in cows increased for FO and FOCO diets with the highest level for FO diet and cows intake of C22:5 and DHA increased for all oil supplemented diets which was an expected response when fish oil was included in diets because of high concentration of these FAs in fish oil containing diets. The intake of C20:4 increased in all oil supplemented diets in comparison with the control diet, but FOCO had the highest intake of this FA among supplemented diets. Cows fed FOCO and CO diets had the highest intake of cis-9 C18:1 and CO diets have shown the highest intake of cis-11 C18:1 among the diets. Loor and Herbein (2003) have reported the similar FAs intake in cows fed diet containing high oleic and high linoleic sunflower oils (2.5% DM). But as reported by these researchers, DMI in cows fed with supplemented diets were similar in all treatments.

Digestibility of OM and NDF decreased ($P<0.05$) in FO diet, but fat, CP and ADF digestibility were not affected by diets (Table 4). The digestibility of structural carbohydrates often decreases in oil containing diets, 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 (Hristove *et al.*, 2005) and the basal diet (Ueda *et al.*, 2003), especially forage to concentrate ration (Sutton *et al.*, 1983). 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 Chiilard, 1997) with around 35% concentrate ration in diet, a positive effect of n-3 FAs 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

Table 3: Least square means of milk yield and composition and blood metabolites for lactating dairy cows fed experimental diets.

Parameter	Treatments[1]				SEM[2]	p
	Control	FO	FOCO	CO		
Milk yield						
Actual, kg/d	34.08	33.84	34.55	33.90	0.86	ns
FCM[3]4%, kg/d	31.24[a]	23.63[b]	27.03[b]	27.06[b]	1.16	**
ECM[4], kg/d	33.34[a]	26.81[b]	29.96[b]	29.69[b]	1.01	**
Milk components						
%Fat,	3.43[a]	2.32[b]	2.47[b]	2.67[b]	0.13	**
Fat yield, Kg/d	1.17[a]	0.68[b]	0.87[b]	0.90[b]	0.05	**
%Protein,	2.99	2.92	2.89	2.86	0.07	ns
Protein yield, Kg/d	0.98	0.97	1.00	0.96	0.02	ns
%Lactose,	4.38	4.39	4.33	4.27	0.05	ns
Lactose yield, Kg/d	1.49	1.47	1.49	1.44	0.03	ns
%SNF,	7.98	8.01	7.92	7.83	0.10	ns
SNF yield, Kg/d	2.70	2.68	2.73	2.65	0.05	ns
Serum Metabolites						
Glucose, mg/dl	63.75	60.62	61.87	63.75	2.22	ns
Cholesterol, mg/dl	192.87	211.75	207.5	194.37	8.75	ns
Triglyceride, mg/dl	9.57	10.19	10.12	11.38	0.99	ns
Insulin, miclu/ml	18.6	13.65	13.66	13.36	4.96	ns
SUN, mg /dl	18.87	17.62	18.00	18.12	0.73	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.

[3] FCM4%= 0.4× [milk yield(kg)]+15×[fat yield(kg)].

[4] Energy Corrected milk= [7.2×protein yield (kg) +12.95×fat yield(kg)+0.327×milk yield(kg)]

ns = not significant

*P<0.05, **P<0.01

[a,b,c] Row means differ significantly.

Table 4: Means of nutrient intake (kg/d) and nutrient digestibility (%) in cows fed experimental diets.

Parameter	Treatments[1]				SEM[2]	p
	Control	FO	FOCO	CO		
Intake , Kg/d						
DM	24.92[a]	22.21[b]	24.61[a]	24.86[a]	0.61	*
OM	23.00	20.39	21.07	21.91	0.72	ns
NDF	7.98	7.06	7.45	7.96	0.45	ns
ADF	4.76	4.05	4.30	4.43	0.24	ns
Fat	0.79[a]	1.03[b]	1.15[b]	1.13[b]	0.01	***
CP	4.17[a]	3.51[b]	3.68[a]	3.91[a]	0.15	*
Digestability,						
OM	65.58[a]	60.62[b]	62.98[a]	62.33[a]	1.05	*
NDF	61.81[a]	51.55[b]	52.22[a]	53.89[a]	2.52	*
ADF	43.63	42.11	42.42	43.72	1.05	ns
Fat	65.43	67.76	69.57	68.9	1.4	ns
CP	65.79	66.56	64.94	65.91	1.98	ns
Rumen parameter						
N-NH3, m mol^{-1}	12.92	10.80	11.45	10.39	0.66	ns
PH	6.68	6.84	6.75	6.61	0.12	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.

Table 5: FA intake in cows fed control, fish oil (FO), fish oil with canola oil (FOCO), or canola oil (CO) diet(g/100g FAs).

FAs	Treatments[1]				SEM[2]	P value
	Control	FO	FOCO	CO		
C14:0	0.1[a]	0.3[b]	0.2[b]	0.09[a]	0.005	***
C16:0	3.38[a]	3.57[ac]	3.16[ba]	2.83[d]	0.08	**
C16:1	0.04[a]	0.04[a]	0.02[bc]	0.01[bc]	0.002	***
C18:0	0.75	0.83	0.82	0.81	0.01	ns
C18:1 c9	5.77[a]	5.29[a]	7.46[b]	8.6[c]	0.19	***
C18:1 c11	0.64[a]	0.52[b]	0.67[ac]	0.73[c]	0.01	***
C18:2 c9c12	8.14[a]	4.95[b]	5.92[c]	6.62[d]	0.15	***
C20:4	0.46[a]	0.7[b]	0.91[c]	0.78[b]	0.01	***
C20:5 EPA	0.43[a]	1.78[b]	0.78[c]	0.51[a]	0.02	***
C22:5	0.32[a]	0.24[b]	0.27[b]	0.47[c]	0.09	***
C22:6 DHA	0.33[a]	0.64[b]	0.61[b]	0.47[c]	0.01	***

[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<0.01, ***P<0.0001
[a,b,c] Row means differ significantly.

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 FAs of oil supplements increase negative effects of them (Hristove *et al.,* 2005). Digestibility of OM in total tract 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 to 11% of DM) had no effects on total tract digestibility in sheep (Wachira *et al.*, 2000), or in lactating dairy cows (Petit *et al.*, 2002). 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 *et al* (2002) 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.

Rumen parameter

Diet supplementation with fish oil and canola oil had no significant effect on rumen PH and N-NH_3 (Table 3), although rumen N-NH_3 concentration tend to be lower for cows fed supplemented diets (P=0.07). Results from previous reports however, are variable. The overall mean concentration of rumen N-NH_3 and pH were not affected when Jet-sploded canola seed (khorasani *et al.*, 1998), different level of fish oil and extruded soybean (Abughazaleh *et al.*, 2002) and ground canola seed (Chichlowski *et al.*, 2005) was included in diets. Unsaturated oil has negative effects on rumen protozoa population and will decrease proteolysis of microbial protein which finally leads to improve efficiency of microbial protein synthesis (Hristove *et al.*, 2005). Oldick and Firkins (2000) also observed a linear decrease in ruminal protozoa with increasing the degree of unsaturation in dietary FAs. Decreased protozoal papulation in the rumen are usually associated with lowered N-NH_3 concentrations (Williams and Coleman, 1992). Moreover, it seems the magnitude of the antiprotozoal properties of feed oils depend on the degree of unsaturation of the FAs (Hristove et al., 2005). However, supplementing high fiber and high concentrate diets with linseed oil (Ueda *et al.*, 2003) both increased ruminal N-NH_3 concentration, but rumen pH was not affected by diets. Including saturated, intermediate saturated and unsaturated FAs had no effects on ruminal PH (Harvantine and Allen, 2006). Similar to previously reported decreasing effect of unsaturated oil on rumen N-NH_3 concentration, supplementing diets tend to have lower concentration of rumen N-NH_3

Generally, based on results of the current study in comparison with separately added fish oil and canola oil in diets, combination of fish oil and canola oil increase the energy density of Holstein dairy diets in early lactation with out negative effects on milk production and components, nutrient intake and digestibility.

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