Effects of Fish Oil, Safflower Oil and Monensin Supplementation on Performance, Rumen Fermentation Parameters and Plasma Metabolites in Chall Sheep

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Abstract: Thirty-two lactating Chall ewes were assigned to eight groups and received one of eight dietary treatments for ten weeks as follows: control diet (CON); a fish oil-supplemented diet (20 g/kg dry matter intake (DMI); FO); a safflower oil-supplemented diet (20 g/kg DMI; SO); a monensin-supplemented diet (15 mg/kg DMI; MO); a diet supplemented with fish oil (20 g/kg DMI) and safflower oil (20 g/kg DMI; FS); a diet supplemented with fish oil (20 g/kg DMI) and monensin (15 mg/kg DM;I FM); a diet that contained safflower oil (20 g/kg DMI) and monensin (15 mg/kg DMI; SM); and a diet that contained fish oil (20 g/kg DMI), safflower oil (20 g/kg DMI) and monensin (15 mg/kg DMI; FSM). During the experimental period, ewes were kept in individual pens and the amounts of feed that were offered to individual ewes were recorded daily. Milk samples were collected weekly and analyzed for their composition. Rumen fluid and plasma samples were obtained from each ewe on the final day of the trial and stored at -20˚C before analysis. Results showed that the SO diet had no significant effect ($p< 0.05$) on DMI, However, other diets reduced the DMI significantly. Milk yield was significantly lower for ewes that were fed diets supplemented with monensin compared to the other groups ($p<0.05$). The milk fat percentage was relatively low for all treatments, although only FS, FM and FSM treatments were significantly different from the control group. The yield of milk fat was significantly reduced in the MO, FS, FM, SM and FSM groups. The percentage of protein in milk was significantly reduced in the FS and FSM diets. However, the milk protein yield was reduced significantly as a direct result of the decreased milk yield in ewes that were fed the MO, FS, FM, SM and FSM diets. The MO diet increased significantly the concentration of urea nitrogen in milk. Fish oil and safflower oil-supplemented diets with or without monensin resulted in decreased numbers of infective protozoa, and a decreased acetate-to-propionate ratio in rumen fluid when compared to the CON group. The concentrations of plasma glucose and urea were not affected by any of the treatments, but the plasma concentrations of triglycerides, total cholesterol, and HDL-cholesterol were higher in ewes that consumed the oil-containing diets than the other groups. It was concluded that dietary supplementation with a combination of fish oil, safflower oil and monensin could alter the composition of milk from ewes and, in particular, cause a reduction in the percentage of fat in the milk. This may make this milk more suitable for human consumption.

Keywords: Milk, ewe, fish oil, safflower oil, monensin.

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Introduction

Relative to bovine and caprine milk, ovine milk is characterized by its higher content of total solids and fat, which makes it ideal for cheese production (Haenlein, 2001). However, the high fat content in the milk of sheep might limit the demand for milk and milk products by health conscious consumers (Zhang *et al.*, 2006a). In recent years, considerable attempts have been made to alter the composition of milk to make it more suitable for human consumption. Modification of the diet of ewes shows the most promise as a management strategy to change the composition of milk in the short-term (Cant *et al*., 1997). Dietary fats, which are rich in polyunsaturated fatty acids, have been used to manipulate the fatty acid composition of milk from ewes by an improvement in the unsaturated: saturated fatty acid ratio (Zhang *et al*., 2006a). Most studies regarding the effects of supplementation with fats in ruminants have involved dairy cows. However, data is limited on giving feed supplementation with dietary fats, such as fish oil and safflower oil, to lactating ewes.

Fish oil and safflower oil would be a good choice for feed supplementation from the point of view of consumers as both are rich in polyunsaturated fatty acids. Fish oil is a source of C20:5 and C22:6 fatty acids, which are n-3 fatty acids, whereas safflower oil have plentiful amount of linoleic acid (LA), which is n-6 fatty acid. Previous studies have shown that n-3 fatty acids may help to prevent coronary heart disease (Donovan *et al*., 2000; Shingfield *et al*., 2003).The n-3 fatty acids such as eicosapentaenoic acid (EPA [(C20:5 (n-3)]) and docosahexaenoic acid (DHA [(C22:6 (n-3)]) are present in substantial quantities in fish oil. Therefore, the inclusion of fish oil in the diet of ruminants has increased the concentration of these long-chain polyunsaturated fatty acids in milk fat that may be beneficial for human health (Shingfield *et al*., 2003; Lee *et al*., 2005). The supplementation of feeds with high amounts of long-chain fatty acids has been shown to increase milk fat and inhibit de novo synthesis of short- and medium-chain fatty acids in bovine mammary glands (Palmquis *et al*., 1993). The evidence is limited with regards to the metabolism of fish oil in the rumen, but less than 50% of the lipids in fish oils are likely to be hydrolyzed in the rumen, compared to more than 90% of plant oils (Donovan *et al*., 2000). Safflower oil was chosen in this study because of its high level of cis-9, cis-12 18:2, which may represent a feasible dietary source of LA for livestock. Lambs that were fed with safflower seeds had increased unsaturated fatty acids and concentrations of cis-9, trans-11 conjugated linoleic acid (CLA) in their muscle tissue (Bolte *et al*., 2002; Kott *et al*., 2003; Boles *et al*., 2005).

Monensin, which is one of monocarboxylic acid ionophore antibiotics, has been known to inhibit the growth of Gram-positive bacteria that produce hydrogen and alter lipid metabolism in the rumen (Wang *et al*., 2005). Previous studies have shown that ionophores interfere with the biohydrogenation of cis-9, cis-12 18:2 and caused a reduction in the extent of cis-9, cis-12 18:2 biohydrogenation with an accumulation of intermediate products, including CLA (Fellner *et al*., 1997; Bell *et al*., 2006). Monensin may increase the protein content in the milk of dairy animals (Brown and Hogue, 1985; Lynch *et al*., 1990). Monensin can also cause a reduction in the percentage of milk fat (Cant *et al*., 1997; Sauer *et al*., 1998). Ionophores disrupt ruminal biohydrogenation in a similar manner to unsaturated fat supplements, and monensin supplementation of feeds enhanced the levels of LA and trans fatty acids in the milk of lactating cows (Jenkins *et al*., 2003).

The objective of this study was to evaluate the effects of supplementing the feeds of lactating ewes with fish oil and safflower oil with or without monensin. Data was collected with regards to their milk yield and composition, their plasma metabolites and ruminal parameters.

Materials and Methods

Animals and diets

Thirty-two lactating Chall ewes (2, 3 or 4 yrs old and 50 ± 1.5 kg body weight [BW]) were assigned to one of eight groups (n = 4 in each group), and were

balanced for their milk production at 10 and 14 d after lambing. Each group was randomized to be fed one of eight different dietary treatments (Table 1). The diets with a 52-55: 48-45, forage:concentrate ratio (dry matter intake (DMI) basis) were formulated to meet the nutritional requirements of a 50 kg ewe in the first six to eight weeks of lactation with twin suckling lambs (CNCPS, 2001). The experimental diets consisted of: 1) a control diet, without supplementation (CON); 2) a diet supplemented with fish oil (20 g/kg of DMI; FO); 3) a diet supplemented with safflower oil (20 g/kg of DMI; SO); 4) a diet supplemented with monensin (15 mg/kg of DMI; MO); 5) a diet supplemented with fish oil (20 g/kg of DMI) and safflower oil (20 g/kg of DMI; FS); 6) a diet supplemented with fish oil (20

Table 1: Ingredient and chemical composition of experimental diets.

	Treatment diets[1] (on DM basis)							
Items **Diet Ingredients (g/kg)**	**CON**	**FO**	**SO**	**MO**	**FS**	**FM**	**SM**	**FSM**
Alfalfa hay	339.9	373.0	373.0	339.9	321.3	372.9	372.9	321.3
Corn silage	105.1	83.4	83.4	105.0	110.3	83.4	83.4	110.3
Barley straw	76.5	86.8	86.8	76.5	118.2	86.7	86.7	118.2
Barley grain	205.5	146.8	146.8	205.4	86.3	146.8	146.8	86.3
Cottonseed meal	94.5	122.8	122.8	94.5	135.3	122.7	122.7	135.3
Beet pulp	85.4	82.4	82.4	85.0	89.3	82.4	82.4	89.3
Soybean meal	71.4	62.2	62.2	71.4	75.1	62.2	62.2	75.1
Fish oil	0	20.0	0	0	20.0	20.0	0	20.0
Safflower oil	0	0	20.0	0	20.0	0	20.0	20.0
Monensin	0	0	0	0.015	0	0.015	0.015	0.015
Dicalcium phosphate	9.1	9.2	9.2	9.1	9.6	9.3	9.3	9.5
Limestone	3.7	4.8	4.8	3.7	4.9	4.8	4.8	4.9
Mineral-vitamin mix[2]	4.5	4.7	4.7	4.7	4.9	4.7	4.7	4.9
Salt	4.6	3.9	3.9	4.7	4.8	4.0	4.0	4.8
Chemical compositions (%)								
CP (g/kg)	16.20	16.25	16.28	16.20	16.19	16.23	16.26	16.18
EE	2.20	4.21	4.17	2.23	6.25	4.20	4.19	6.23
NDF	39.31	40.53	40.15	38.94	40.80	40.62	40.20	40.83
ADF	24.11	25.38	25.35	23.90	26.33	25.46	25.20	26.35
Ash	8.4	8.7	8.5	8.5	8.8	8.9	8.8	8.8
Ca	0.89	0.9	0.88	0.91	0.93	0.90	0.87	0.93
P	0.42	0.43	0.43	0.42	0.44	0.43	0.43	0.45
ME, (Mcal/kg DM)	2.271	2.313	2.313	2.271	2.385	2.312	2.311	2.385
Dietary forage (%)	52.2	54.3	54.3	52.2	55.0	54.3	54.3	55.0

[1]CON: control; FO: control supplemented with fish oil; SO: safflower oil; MO: monensin; FS: fish oil and safflower oil; FM: fish oil and monensin; SM: safflower oil and monensin; FSMN: fish oil, safflower oil and monensin.

[2]Contained Vitamin A (400000 IU/kg), Vitamin D3 (100000 IU/kg), Vitamin E (200 IU/kg), Calcium (180 g/kg); Phosphate (70 g/kg), Mg (30 g/kg), Na (50 g/kg), Fe (4 g/kg), Cu (0.3 g/kg), Zn (3 g/kg), Mn (5 g/kg), I (0.1 g/kg), Co (0.1 g/kg), Se (0.02 g/kg).

g/kg of DMI) and monensin (15 mg/kg of DMI; FM); 7) a diet supplemented with safflower oil (20 g/kg of DMI) and monensin (15 mg/kg of DMI; SM); and 8) a diet supplemented with fish oil (20 g/kg of DMI), safflower oil (20 g/kg of DMI) and monensin (15 mg/kg of DMI; FSM).

During the experimental period, ewes were kept in individual pens, and water was available freely at all times. The diets were fed with three meals of equal size every day of the trial at 08:00 h, 12:00 h, and 16:00 h as Total Mixed Ration (TMR) for 12 weeks, which allowed two weeks for dietary adaptation and ten weeks for data collection. The amounts of the feeds that were offered and the amount of daily remained feed (orts) for each ewe were recorded daily for each individual ewe, and the feed orts were adjusted to maintain between 5% to 10% of intake on as fed basis.

Sampling and chemical analysis

The TMR offered and orts were measured on a daily basis. Feed and orts were taken once weekly during the experimental period and analyzed for the levels of DMI, CP, EE, Ash (AOAC 2000) NDF, ADF (Van Soest *et al*. 1991). The volume of milk production was recorded weekly for ten weeks. It was measured over a six hour period in a day by the use of combined hand-milking and the use of oxytocin (2 IU of oxytocin, Scanpharm Denmark Company) as described by Perez Alba *et al.* (1997), and the daily milk yield was then calculated. Milk samples were then taken from each ewe and combined with preservative (dichromate potassium) before being stored at 4˚C until analysis. The samples were then analyzed for the levels of fat, protein, lactose, total solids (TS), and solids that were not fats (SNF) using a Milko-Scan 133 B (Foss Electric, Hillerod, Denmark) and somatic cell count (SCC) using a Fossomatic 90.

Approximately 300 ml of ruminal fluid was collected from each ewe at 07:00 h on the last day of week ten prior to the morning feed via a stomach tube (Jones *et al*., 2000). The pH of the samples was measured immediately after the sampling, and then a 100 ml of rumen fluid from each animal was strained through four layers of cheesecloth. Three milliliters of metaphosphoric acid (250 g/l) were added to 15 ml from each of the ruminal fluid samples, and then these were stored at –20˚C before analysis of the total volatile fatty acids (VFA). Additionally, 20 ml of hydrochloric acid (HCl; 0.2 N) was added to 20 ml from each of the ruminal fluid samples and stored at -20˚C for free ammonia (NH3–N) analysis (Heldt *et al*., 1999). The samples were thawed and the concentration of NH3-N in ruminal fluid was determined with the use of a titration method, as described by Crooke and Simpson (1971). The concentration of VFAs was determined as described by Ottenstein and Batler (1971) with a gas chromatograph (Philips PU 4410). Four-Methyl Valeric Acid (Sigma) was used as the internal standard. Briefly, 1 ml of the ruminal fluid sample and 60 µl of the internal standard were transferred into a 15 ml glass test tube, and these were centrifuged at 3000 × g for 20 min at 4˚C. The upper layer was then transferred into a clean 15 ml glass tube with a Pasteur pipette, and 8 µl was injected into the gas chromatograph. The initial column temperature was 80˚C, which was then increased at a rate of 10˚C/min to 180˚C. The injector and detector temperatures were maintained at 200˚C. Hydrogen was the carrier gas.

Ruminal fluid samples from ewes in each of the treatment groups were also used to count the number of protozoa; 10 ml of strained ruminal fluid were mixed with 10 ml of 50% formalin (18.5% formaldehyde, V/V) and stored at 10˚C before estimating the protozoal population (Dehority, 2003). One milliliter of the preserved samples was the pipetted into a 16 × 150 mm test tube, two drops of Brilliant green dye were added, the tubes were mixed and then allowed to stand overnight. Each sample was then counted twice to determine the mean average of protozoa in each sample.

Blood samples (50 ml) were collected in heparin-containing tubes from the jugular vein before the morning feed on the last day of week ten in order to estimate the plasma concentration of

metabolites (Chilliard and Ottou, 1995) Blood was centrifuged at 3000 × g at 4˚C for 15 min and plasma was harvested and stored at -20˚C until they were analyzed for metabolites (Loor *et al*., 2005). Plasma concentration of glucose, triglyceride, total cholesterol, high–density lipoprotein cholesterol (HDL-cholesterol), and low-density lipoprotein cholesterol (LDL-cholesterol) were determined with the enzymatic, colorimetric-endpoint method and the enzymatic, fixed-time method using commercial kits (Pars Azmun Iran Company) with a spectrophotometer (Shimadzu 2100, VIS spectrophotometer Shimadzu, Kyoto, Japan).

Statistical analysis

The data of milk yield and composition were analyzed according to a completely randomized factorial design with time (weeks) as a repeated measure using the MIXED procedure of the Statistical Analyzer Software (SAS, 1996). The model for statistical analyses of dry matter intake, milk yield, milk component percentages and yields included: covariate adjustment, supplement (FO, SO, MO), week, supplement × week interaction, and residual error. Observations during the first two weeks prior to initiation of the 10-wk lactation trial were used as covariates for the respective and least square means (LSM) of the covariates. Data were reported as LSM with a pooled standard error (SE). The LSM were separated by least squares difference. Overall differences between treatment means were considered significant when the p-value was less than 0.05.

Ruminal fluid measurements and plasma metabolites were analyzed using the general linear model (GLM) procedure of the SAS program (SAS, 1996). Results were reported as LSM with pooled SE. The differences between observations were designated as significant when the p-value was less than 0.05.

Results

Dry matter intake (DMI), milk yield and composition

The effect of diets on the DMI is presented in Table 2. The supplementation of the control diet with fish oil significantly reduced the DMI ($p<0.05$). Supplementation with safflower oil decreased DMI, although this difference was not significant between SO and CON treatments, and only a slight reduction in DMI was observed for the SO diet when compared with the CON group. The reduction in DMI was higher with the FO diet compared to the SO diet. Addition of fish oil to safflower oil (FS diet) produced a greater decrease in DMI, and this reached significance for FS compared with both the CON and SO diets (1.82 kg/d vs. 2.36 kg/d and 2.07 kg/d, respectively). The drop in DMI appeared to be more pronounced for the diets that contained monensin than for the control diet, and this reached significance for the MO, FM, SM, and FSM diets when compared with the CON (1.88 kg/d, 1.62 kg/d, 1.87 kg/d and 1.58 kg/d vs. 2.36 kg/d, respectively). The highest decrease in DMI was observed with the FSM and FM diets when compared with the CON diet.

Milk yield and composition data are shown in Table 2. Milk yield declined for all groups when compared with the CON diet, although it was significantly lower ($p<0.05$) in ewes that were fed with MO, FM, SM, and FSM diets than those fed with the CON diet (1070.9 g/d, 926.9 g/d, 1123.2 g/d and 1072.3 g/d vs. 1404.4 g/d, respectively). However, no significant differences were found between the milk yield and composition for the FO, SO, FS diets compared with the CON diet.

The percentage of milk fat was relatively low for all treatments (Table 2), but only the FS, FM, and FSM diets were significantly different when compared with the CON diet (8.42%, 7.72% and 8.09% vs. 9.55%, respectively). Due to the decrease in the content of milk fat, the yields of fat were also significantly reduced for ewes that were fed with the MO, FS, FM, SM, and FSM diets when compared with the CON diet group (92.73 g/d, 102.17 g/d, 71.85 g/d, 104.09 g/d and 85.06 g/d vs. 134.55 g/d, respectively). The fat content of milk was lower in ewes that were fed with diets containing monensin

Table 2: Dry matter intake, milk yield and composition.

	Treatment diets[1]								
Items	**CON**	**FO**	**SO**	**MO**	**FS**	**FM**	**SM**	**FSM**	**SE**
DMI, (kg/d)	2.36^{a}	2.07^{bc}	2.22^{ab}	1.88^{cd}	1.82^{cd}	1.62^{d}	1.87^{cd}	1.58^{d}	0.07
Milk yield, (g/d)	1404.4^{a}	1248.6^{ab}	1271.4^{ab}	1070.9^{bc}	1178.7^{abc}	926.9^{c}	1123.2^{bc}	1072.3^{bc}	71.50
Fat, (%)	9.55^{a}	9.41^{ab}	9.48^{ab}	8.74^{abc}	8.42^{bc}	7.72^{c}	9.28^{ab}	8.09^{c}	0.65
Fat yield, (g/d)	134.55^{a}	118.57^{ab}	120.91^{ab}	92.73^{cd}	102.17^{bc}	71.85^{d}	104.09^{bc}	85.06^{cd}	4.97
Protein, (%)	5.31^{ab}	5.45^{a}	5.18^{ab}	5.17^{ab}	4.76^{b}	5.20^{ab}	5.23^{ab}	4.78^{b}	0.15
Protein yield, (g/d)	74.13^{a}	67.35^{ab}	65.22^{abc}	54.20^{cd}	55.97^{cd}	47.47^{d}	58.85^{bc}	50.68^{cd}	2.41
Lactose, (%)	5.36	5.27	5.38	5.28	5.32	5.45	5.25	5.51	0.06
Lactose yield, (g/d)	74.45^{a}	64.97^{abc}	68.73^{ab}	56.75^{bc}	62.95^{abc}	50.66^{c}	59.35^{bc}	59.68^{bc}	3.50
Total solids, (%)	20.42	20.87	20.83	19.92	19.35	19.21	20.65	19.22	0.52
Solids (not fats), (%)	11.29	11.23	11.18	11.06	10.80	11.26	11.09	10.99	0.14
Milk urea N, (mg/dl)	16.97^{b}	18.22^{ab}	17.80^{ab}	19.97^{a}	17.78^{ab}	18.50^{ab}	17.98^{ab}	18.36^{ab}	0.79
SCC, (× 10³/ml)	106.75	125.75	133.25	104.13	154.88	100.25	142.0	152.88	28.48

$^{a, b, c, d}$ Values within a row with different superscripts are significantly different ($p<0.05$)

[1] CON: control; FO: control diet supplemented with fish oil; SO: safflower oil; MO: monensin; FS: fish oil and safflower oil; FM: fish oil and monensin; SM: safflower oil and monensin; FSMN: fish oil, safflower oil and monensin.

than in ewes that had no monensin in their diets, and the combination of monensin with fish oil caused significant decrease in the percentage of milk fat and the milk yield. However, addition of monensin to the safflower diet did not have significant effect on these parameters.

The percentage of milk protein was significantly reduced (Table 2) for ewes fed with the FS and FSM diets compared with the FO diet (4.76% and 4.78% vs. 5.45%, respectively). However, no significant differences were observed between the experimental diets compared to the CON diet ($p<0.05$). Our data showed that fish oil and safflower oil together led to the significant decrease in the percentage of milk protein and yield. The percentage of milk protein was not affected by the MO diet.

Among the experimental diets used in the present study, the milk protein yield was significantly decreased ($p<0.05$) by the MO, FS, FM, SM and FSM diets when compared with the CON diet (54.20, 55.97, 47.47, 58.85 and 50.68 vs. 74.13 gram/day, respectively).

The percentage of lactose, TS and SNF in milk were not significantly different between the treatments in this study. However, the milk lactose yield was significantly decreased ($p<0.05$) by diets that contained monensin (MO, FM, SM and FSM) compared with the CON diet because of the decreased milk yield of ewes that were fed with these diets (56.75, 50.66, 59.35 and 59.68 vs. 74.45 gram/day, respectively). No difference occurred between the other diets.

The milk urea nitrogen (N) concentration was increased by monensin supplementation (Table 2), although only MO diet increased significantly the concentration of milk urea N compared to the CON diet (19.97 vs. 16.97 mg/dl). The supplementation of oils with or without monensin also resulted in increased milk urea N concentrations, but these effects were not statistically significant. No significant differences ($p>0.05$) were observed in the somatic cell count (SCC) analysis between treatments.

Rumen parameters

Protozoa numbers, pH and NH3-N concentration

Feeds that were supplemented with oils with or without monensin significantly decreased ($p<0.05$) the total number of protozoa in the rumen fluid samples from approximately 2.5×10^6/ml in the CON diet to slightly less than 6×10^5/ml in theFM and FSM diets (Table 3). The effects on this parameter in all of the experimental diet groups were significantly different when compared to the controls. In the present study, supplementation of diets with oils and monensin did not significantly affect the pH of the rumen (Table 3). The concentration of ruminal NH3-N was decreased by the treatment diets, and this tended to be lower in ewes that were fed with diets that contained oil and monensin together (Table 3).

VFA concentration

The total concentration of VFAs (mmol/L) was not significantly different between the groups with different diets. However, the supplementation of diets with oils or monensin resulted in a reduction in the concentration of VFA when compared with the ewes in the control diet (Table 3). The combination of the different oils with monensin caused a greater reduction in the concentration of VFA relative to their use alone. Dietary supplementation with safflower oil in both the SO and FS diets significantly reduced ($p<0.05$) the concentration of acetic acid in rumen fluid. The addition of fish oil and monensin also reduced the acetic acid concentration. The acetic acid concentrations were lower numerically in diets with safflower oil than other treatments. The significant increase in the concentration of propionate ($p<0.05$) when oils or monensin was added. Our data showed that adding fish oil and safflower oil together may result in a higher increase in the concentration of propionate compared the controls (23.34% vs. 9.73%)($p<0.05$). We observed that the concentration of propionate was higher in diets with safflower oil compared to diets without it. The concentration of butyrate decreased when fish oil, safflower oil or monensin was added to the diets, but this reduction was not significant. Dietary supplementation had no significant effect on the proportion of valeric acid, but the level of isovalerate was significantly higher ($p<0.05$) in ruminal fluid from ewes fed with the FO and MO diets compared with controls (2.70% and 1.50% vs. 4.65%, respectively) ($p<0.05$). The SO diet did not affect the concentration of isovaleric acid, but the cumulative interaction of monensin and oils were observed in this research. The concentration of isovaleric acid decreased ($p<0.05$) when diet was supplemented with fish oil or safflower oil withdietary supplementations with oils or monensin resulted in a significant reduction ($p<0.05$) in the acetate-to-propionate ratio (A:P) compared with the controls. The lowest ratio was observed significantly in the FSM diet (3.07 vs. 7.49) ($p<0.05$).

Plasma metabolites

The effects of the different diets on the levels of plasma metabolites are presented in Table 4. Plasma concentrations of glucose and urea were not significantly altered. The addition of oil increased the plasma concentrations of total cholesterol and triglyceride ($p<0.05$). Plasma concentrations of total cholesterol and triglyceride were the highest ($p<0.05$) after ewes were fed with the FSM diet compared to the CON diet (82.68 mg/dl and 46.12 mg/dl vs. 56.39 mg/dl and 39.11 mg/dl, respectively). No significant difference was found between fish oil and safflower oil on the plasma concentrations of total cholesterol and triglyceride. The addition of monensin had no significant effect on the plasma concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. The concentrations of HDL-cholesterol and LDL-cholesterol were greater ($p<0.05$) in ewes fed with diets supplemented by oils than the diets without oils.

Discussion

Dry matter intake (DMI)

The decrease in DMI has been attributed to a decrease in the degradation of fiber and its

Table 3: pH, protozoa numbers and mean concentration (Mm/l) of VFA in the ruminal fluid of ewes fed with experimental diets.

	Treatment diets[1]								
Constituents	**CON**	**FO**	**SO**	**MO**	**FS**	**FM**	**SM**	**FSM**	**SE**
Ruminal fluid, (pH)	6.81	6.91	6.98	7.04	7.13	7.17	7.23	7.09	0.20
Protozoa number, ($\times 10^6$)	2.532^{a}	1.151^{bc}	1.525^{b}	1.220^{bc}	1.106^{bc}	0.530^{d}	1.034^{c}	0.547^{d}	0.16
NH3-N, (mg/100 ml)	18.53^{a}	13.47^{b}	14.80^{b}	13.95^{b}	12.59^{b}	12.40^{b}	13.86^{b}	12.81^{b}	0.80
Total VFA, (mM/l)	57.48	50.95	56.33	56.70	51.03	44.83	40.60	42.43	7.12
Acetic acid, (%)	74.33^{a}	69.32^{ab}	64.02^{b}	67.64^{ab}	69.93^{ab}	70.96^{ab}	67.42^{b}	67.43^{b}	2.31
Propionic acid, (%)	9.73^{c}	18.04^{b}	19.04^{ab}	19.83^{ab}	19.43^{ab}	18.29^{b}	21.53^{ab}	23.34^{a}	1.69
Butyric acid, (%)	13.47^{a}	10.18^{ab}	12.68^{a}	10.47^{ab}	7.65^{b}	8.40^{b}	10.14^{ab}	7.75^{b}	1.43
Iso-valeric acid, (%)	4.65^{a}	2.70^{bc}	3.97^{ab}	1.50^{c}	1.72^{c}	1.55^{c}	1.42^{c}	1.36^{c}	0.69
Valeric acid, (%)	0.68	0.63	0.54	0.40	0.47	0.71	0.65	0.60	0.09
A: P	7.49^{a}	4.02^{b}	3.47^{b}	3.37^{b}	3.90^{b}	3.98^{b}	3.27^{b}	3.07^{b}	0.45

[a, b, c, d] Values within a row with different superscripts are significantly different ($p<0.05$)

[1] CON, control; FO, control supplemented with fish oil; SO, safflower oil; MO, monensin; FS, fish oil plus safflower oil; FM, fish oil plus monensin; SM, safflower oil plus monensin; FSMN, fish oil plus safflower oil plus monensin.

Table 4: Mean concentration (mg/dl) of plasma metabolites in ewes fed with experimental diets.

	Treatment diets[1]								
Items	**CON**	**FO**	**SO**	**MO**	**FS**	**FM**	**SM**	**FSM**	**SE**
Glucose	63.93	64.14	64.94	66.79	67.36	67.75	64.92	67.35	3.44
Triglyceride	39.11^{c}	42.96^{b}	42.84^{b}	39.04^{c}	44.06^{ab}	42.75^{b}	42.86^{b}	46.12^{a}	0.90
Total cholesterol	56.39^{d}	70.15^{b}	69.29^{b}	55.86^{c}	83.05^{a}	67.34^{b}	68.68^{b}	82.68^{a}	3.23
HDL-cholesterol	38.23^{c}	46.35^{ab}	43.46^{ab}	37.63^{c}	51.06^{a}	46.18^{ab}	46.58^{ab}	51.49^{a}	3.52
LDL-cholesterol	18.16^{c}	24.80^{bc}	25.73^{bc}	18.29^{c}	31.09^{a}	22.16^{bc}	23.25^{bc}	31.32^{a}	3.11
Urea	32.20	28.60	29.45	31.65	34.09	29.52	32.22	34.22	2.58

[a, b, c, d] Values within a row with different superscripts are significantly different ($p<0.05$)

[1] CON: control; FO: control diet supplemented with fish oil; SO: safflower oil; MO: monensin; FS: fish oil and safflower oil; FM: fish oil and monensin; SM: safflower oil and monensin; FSMN: fish oil, safflower oil and monensin.

digestibility in the rumen, which alters the ratio of ruminal acetate to propionate. In turn, is due to the direct adverse effect of fats, especially from high in unsaturated fatty acids on ruminal microbes and fermentation(NRC, 2001). Previous researchers have indicated that plant oil or fish oil supplementation of feeds causes a significant inhibition of the digestion of fiber (Jenkins, 1993; 2005). The high concentration of unsaturated fatty Cant *et al.*, 1997; Donovan *et al.*, 2000; He *et al.*, 2005). The high concentration of unsaturated fatty acids in fish oil is likely to be effective inhibitors of fiber digestion in the rumen (Jenkins and Jenny, 1989). Unsaturated fatty acids inhibit cellular respiration and cause lysis of bacterial cells (Galbraith and Miller, 1973b; Donovan *et al.*, 2000). The negative effect of dietary oil has been attributed to four factors (He *et al.,* 2005): 1) the physical

coating of the feed with oil, which restricts the access of microbes to feed particles; 2) the formation of insoluble soaps, which decreases the availability of cations such as calcium; 3) the inhibition of microbial activity; and (4) the toxicity to certain microbes, especially protozoa.

The effect of fat supplementation on the DMI was related to various factors including the volume and degree of the unsaturation of fatty acids, and the type of fat that supplements the diet (NRC, 2001). Fish oil is composed of highly polyunsaturated fatty acids compared with safflower oil, which is a source of polyunsaturated fatty acids (Thomas *et al*., 1997). Therefore, it could be expected that the DMI decreased to a greater extent for fish oil compared to safflower oil. Consistent with this, the addition of fish oil to the safflower oil (FS diet) produced a further decrease in the DMI in our study. A similar effect of safflower oil on DMI has been observed by Bell *et al*. (2006) when diet supplemented with 6% DMI safflower oil. The effect of monensin on DMI has been demonstrated before (Cant *et al*., 1997; Sauer *et al*., 1998; Jenkins *et al*., 2003; Bell *et al*., 2006). Cant *et al*., (1997) reported a decrease in DMI when monensin was used, and this could be related to the fact that monensin is toxic to specific microorganisms in the rumen. In general, this includes those without an outer membrane such as some cellulolytic species. Johnson *et al*. (1988) also found that the combination of fish oil with monensin produced a greater degree in the decrease of DMI in dairy cows relative to their use alone. Cant *et al*., (1997) reported that monensin may have increased the susceptibility of the rumen microbial population to the membrane–disrupting effects of fish oil.

Milk yield and composition

Our results are consistent with the report of Bell *et al*., (2006) who showed that the milk yield decreased in animals fed with a diet that contained safflower oil and monensin during the treatment period. However, Cant *et al*. (1997) observed that there was no effect on the milk yield when fish oil and monensin were added together to the dairy cow diet. The reduction of milk yield in ewes that were fed with diets that contained oils, and fish oil in particular, may be related to a reduction of DMI and a decrease in ruminal function (Gonthier *et al*., 2005; Zhang *et al*., 2006) that were exacerbated by supplementation with monensin (Cant *et al*., 1997). Generally, the response of different species of dairy animals to supplemental fat were different and may be affected by the source of fat, the volume of supplemental fat in the field and the stage of lactation (NRC, 2001).

Milk fat depression is observed commonly when unprotected oil is fed (Cant *et al*., 1997; Donovan *et al*., 2000; Bell *et al*., 2006; Fatahnia *et al*., 2008). Fish oil and other rumen-active fats induced milk fat depression in lactating cows (Baer *et al*., 2001). It is accepted generally that diets with high level of polyunsaturated fatty acids inhibits the de novo synthesis of milk fat, which leads to low milk fat proportion (Zhang *et al*., 2006). The content of milk fat was lower in ewes that were fed diets containing monensin in our study, which was in agreement with the results of Benchaar (2006) who reported that the addition of 350 mg monensin to the diet resulted in the reduction of milk fat. This reduction was associated with a higher level of trans-10 18:1, which is a potent inhibitor of the synthesis of milk fat. The supplementation of dairy cow diets with 14.5 mg/kg monensin had no significant effect on the percentage of milk fat, although monensin only caused a decrease of 7.5%. However, a combination of 2% fish oil and 14.5 mg/kg monensin caused a significant decline in the percentage of milk fat (Cant *et al*., 1997). Bell *et al.* (2006) found no significant difference in the percentage of milk fat and yield when 24 ppm monensin was fed to Holstein cows. However, a combination of 6% safflower oil and 24 ppm monensin caused a significant decline in the both the percentage of milk fat and the milk yield. Our data showed that supplementation with monensin alone caused a slight reduction in the percentage of milk fat compared with the control group (8.74% vs. 9.55%). Bell *et al.* (2006) suggested that dietary supplementation with monensin did not appear to have independent effect

on the percentage of fat in milk. Our data showed that combination of monensin with fish oil caused a significant decrease in the percentage of milk fat and the milk yield, which concurred with previous research (Cant *et al*., 1997). The results of this latter study suggested that the reduction in milk fat could be a result of the modulation of rumen fermentation towards increased levels of propionate and reduced acetate production in the rumen. However, in the present study, the addition of monensin to safflower diet did not have significant effect.

Our results showed that the combination of fish oil with safflower oil and monensin (FSM) resulted in a significant decline in the percentage of milk fat and yield. This may be related to the addition fish oil, which may have altered the environment in the rumen sufficiently for diets that contained safflower oil and monensin to induce a depressant effect on milk fat.

The percentage of milk protein reduced significantly in ewes that were fed FS and FSM diets. Several studies showed that feeding fat to lactating ewes (Kitessa *et al*., 2003 and Zhang *et al*., 2006b) and goats (Mir *et al*., 1999) had either no effect or decreased the protein content of milk of ewes (Rotunno *et al*., 1998; Casals *et al*., 1999; Chiofalo *et al*., 2004). Cant *et al.* (1993, 1997) reported that blood flow to the udder gland was decreased after feeding ruminants with 4% added dietary fat. They hypothesized that this was due to an increased energetic efficiency of milk synthesis, which manifested as a drop in the mammary blood flow to milk volume ratio, resulting in a decreased content of milk protein. Their proposal was supported by the results of this experiment, in which the level of added dietary fat was 4% (2% fish oil and 2% safflower oil). Additionally, the milk yield was not increased in response to the lipids in feed but the protein content was decreased. The FO and SO diets had no significant effect on the percentage of protein in milk, which was in agreement with others (Mir *et al*., 1999; Donovan *et al*., 2000; Zheng *et al*., 2005; Bell *et al*., 2006; Zhang *et al*., 2006). However, our results were different from that of Cant *et al.* (1997), in which the milk protein concentration of cows was significantly reduced by dietary fish oil supplementation. Also, Rotunno *et al.*, (1998), and Casals *et al.,* (1999) found a negative effect of fat supplementation on the percentage of the milk protein of ewes. Wu and Huber (1994) concluded that reduction in milk protein percentage during fat supplementation might be attributed to an increase in the milk yield without an increase in the number of amino acids available to the mammary gland for protein synthesis.

The percentage of milk protein was not affected by the MO diet. This concurs with previous research studies (Sauer *et al*., 1989; Dhiman *et al*., 1999; Bell *et al*., 2006). However, Cant *et al.* (1997) observed a reduction in the milk protein content when monensin alone or monensin with fish oil was added to the diet.

The percentage of lactose, TS and SNF in milk were not significantly different between the treatment groups, which was consistent with previous findings (Cant *et al*., 1997; Mir *et al*., 1999; Zhang *et al*., 2006b; Baer *et al*., 2001; Bell *et al*., 2006).

In the present study, the concentration of urea N in milk was increased by monensin supplementation. A higher milk urea N concentration has been reported when diets were supplemented with monensin (Duffield *et al*., 1998). According to that study, monensin supplementation increased the amount of protein that reached the small intestine and therefore increased the use of Amino Acids (AA) for gluconeogenesis. This increased the amount of deamination and the concentration of blood urea N. Cant *et al*. (1997) reported that monensin increased the escape of dietary undegraded protein from the rumen, and, therefore, it was expected that the levels of ruminal NH3-N and subsequent urea N of milk and plasma would increase. The numerical increase of milk urea N concentration of ewes that were fed diets supplemented with oils may have been due a reduction in the amount of non-fibrous carbohydrates (NFC) in their diets that reduced the energy available for microbial protein synthesis and resulted in the subsequent increase in the concentration of ruminal

NH3-N (Chouinard *et al*., 1998).

In the present study, SCC was not affected by experimental treatments. Similar results were reported by Baer *et al.* (2001) after the inclusion of 2% fish oil in the diet of dairy cows. However, Abughazaleh *et al*., (2003) observed a significant increase in the SCC when the diet of dairy cows was supplemented with 1% fish oil plus 2% fats from high LA sunflower seeds.

Protozoa numbers, pH and NH3-N concentration

Decreased numbers of protozoa in the ruminal fluid of sheep due to dietary supplementation with fats and oils have been reported in several studies (Ikwuegbu and Sutton., 1982; Sutton *et al*., 1983; Broudiscou *et al*., 1994; Ivan *et al*., 2001). Protozoa have a limited ability to take up, assimilate and transform dietary lipid, and a high dietary lipid concentration is toxic to protozoa (Ivan *et al*., 2001).

Ikwuegbu and Sutton (1982), reported that dietary linseed oil supplementation in sheep resulted in reduced fauna and no significant effects on the pH of the rumen. However, Ivan *et al.* (2001) found that the quantity of fauna was reduced after supplementing feeds with sunflower seed oil, but pH was increased significantly and was more stable in sheep fed with the supplemented diet compared to those on the control diet. Supplementation with monensin tended to increase the ruminal pH in our study, although this was not significant. Other studies have reported a similar increase in ruminal pH (Plaizer *et al*., 2000; Benchaar *et al*., 2006) when dairy cows were fed with diet supplemented with monensin.

The lower NH3-N concentration of ewes that were fed with supplemental oils or monensin was most probably due to the reduction of the protozoal population (Table 3). Therefore, this resulted in the reduction of microbial protein proteolysis and the decrease in microbial nitrogen recycling (Broudiscou *et al*., 1994; Ivan *et al*., 2001).

VFA concentration

The results obtained regarding to total VFA concentration (mmol/l) in this study was consistence with previous findings (Jenkins *et al*., 2003; Wang *et al*., 2005; Benchaar *et al*., 2006). However, the supplementation of diets with oils or monensin resulted in a numerical reduction in the concentration of VFA compared with control diet. The addition of fat partially replaces the nonstructural carbohydrates in the feed and so reduces the fermentable carbohydrate available for VFA production, which results in a decrease in the total VFA concentration in the rumen (Chichlowski *et al*., 2005). In the present study, the higher reduction in VFA concentration in the combination of oil with monensin relative to their use alone may be related to a lower DMI in ewes fed from diets that contain oils and monensin together. Dietary supplementation of oils and monensin tended to reduce the concentration of acetic acid, which could be related to the negative effects of unsaturated fatty acids (Jenkins *et al*., 1993) and monensin (Cant *et al*., 1997) on cellulolytic bacteria and a reduction in the digestability of fiber. Fatty acid esters in polyunsaturated fats (such as safflower oil), particularly oleic (C18:1) and linoleic (C18:2), are hydrolyzed and biohydrogenated with very high efficiency (>90%) in the rumen. These tend to modify fermentation patterns strongly in favor of propionate, but fatty acid esters from highly polyunsaturated fats (such as fish oil), are hydrolyzed less efficiently (<35%), particularly due to the presence of 20-carbon polyunsaturated fatty acids. The latter tend to pass through the rumen unmodified (Jenkins, 1993; Donovan *et al*., 2000). Monensin normally provokes a decrease in C2 and an increase in C3 (Cant *et al*., 1997; Wang *et al*., 2005). The results of the present study are also accordance with their findings (Table 3).

The significant increase in the concentration of propionate ($p< 0.05$) when oils or monensin was added is also comparable with the report of Chilliard *et al*. (2000). Harfoot and Hazlewood (1997) hypothesized that the increase in propionate could be related to the reduction of methane production and the conversion of glycerol of triacylglycerols to

propionate. The propionate concentration was higher in diets with safflower oil compared to diets without. This could be related to LA, which is a major constituent of safflower oil that is known to enhance the production of propionate in the rumen production (Tomas *et al*., 1997).

Our results related to butyrate were consistent with previous findings (Ivan *et al*., 2001; Jenkins *et al*., 2003). The decrease of isovaleric could be related to the effects of unsaturated fatty acids on the degradation of amino acids by rumen bacteria (Harfoot *et al*., 1997). The effect of the combination of monensin with oils is in agreement with other findings (Jenkins *et al*., 2003). The effects of oils or monensin on the A:P ratio could be related the fact that unsaturated oils typically interfere with ruminal fermentation, which causes a drop in A:P ratio, and methane production. This is similar to the effects of the ionophores (Jenkins *et al*., 2003). The efficacy of monensin in modifying the A:P ratio has also been shown to vary with diet composition, particularly with reference to the dietary proportion of the concentrate (Benchaar *et al*., 2006). In the current study, the FSM diet contained 45% concentrate compared with 48% in the CON diet (Table 1), which may explain the significant effects of monensin on the A:P ratio.

Briefly, our data showed that the combination of oil and monensin in the diet of ewes causes a greater reduction in the total VFA concentration, A:P ratio, isovaleric acid concentration, and increase in the concentration of propionate relative to their use alone. The effect of safflower oil was great than that of fish oil.

Plasma metabolites

Supplementation of feed with oils does not increase blood glucose (Grummer and Carrol., 1991), although there was a numerical increase in the glucose concentration in experimental diets in our study, which was likely to be due to the increase of the concentration of propionate (Table 3). Propionate is an important substrate for glucose synthesis in the gluconeogenesis pathway in ruminants (McDonald *et al*., 1997). Other studies reported a similar effect to that seen in our study (Chilliard *et al*., 1993; Kumar *et al*., 2004), or a reduction (Chilliard and Ottou, 1995; Simas *et al*., 1995) of plasma glucose, when dairy cows were fed with diets that were supplemented with fat. According to Duffield *et al*. (1998), monensin supplementation increased the amount of protein that reaches the small intestine and the use of AA for gluconeogenesis.

The addition of fat tended to increase plasma concentrations of total cholesterol and triglycerides ($p<0.05$). These results are in agreement with others (Thomas *et al*., 1997) who reported higher cholesterol and triglyceride concentrations after feeding fat to dairy cows. The increase of the plasma concentration of triglycerides can be related to the higher digestibility of unsaturated fats than saturated fats (Nik-Khah *et al*., 2003). This finding could also be related to increase to the synthesis of cholesterol and triglycerides in the epithelium of the small intestine and liver cells, and the increase of the absorption of these fats from the small intestine after dietary supplementation of fat (Demeyer *et al*., 1999; Chichlowski *et al*., 2005).

Rumen bacteria and protozoa hydrolyze complex lipids into their constituent long-chain fatty acids, sugars, organic bases, and glycerol. Thus, the rumen is the primary site of complex lipid hydrolysis rather than the small intestine. The higher serum levels of triglycerides indicated that the epithelium of the small intestine is capable of absorbing the dietary fatty acids postruminally and incorporating them into triglyceride lipoproteins (Chichlowski *et al*., 2005). Our results are in agreement with others (Grummer *et al*., 1991; Thomas *et al*., 1997) who reported that oils composed of predominantly polyunsaturated fatty acids have also been shown to increase the serum concentrations of lipoprotein cholesterol.

Briefly, our data showed the addition of oil tended to increase plasma concentrations of total cholesterol and triglycerides, but no significant difference between the effect of fish oil and safflower

oil was found. The addition of monensin had no significant effect, but the combination monensin with oils did result in a significant effect.

Implications

The data of this study indicated that the combination of fish oil and monensin had more negative effects on the milk yield and composition of ewe milk than the combination of safflower oil and monensin. However, dietary supplementation with fish oil, safflower oil and monensin together had a lower negative effect on the milk yield with an overall decreased content of milk fat. All of the treatments resulted in a significant decrease in the numbers of protozoa compared with the control diet, but the reduction was higher when monensin was included in the treatments. It can be concluded that the combination of both oils and monensin may be used as a nutritional strategy to decrease the fat content of milk from ewes, which could make this more suitable for human consumption. Dietary supplementation with fish oil, safflower oil and monensin together should be recommended because of the greater effect on the concentration of HDL-cholesterol.

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