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Effects of unprotected and calcium salts of palm and linseed oils on feed intake, total tract digestibility, ruminal degradation and fermentation in lambs

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Abstract The objective of present study was to assess the effects of unprotected and calcium salts of oils on total tract digestibility, ruminal degradation and fermentation in lambs. Four lambs fitted with ruminal fistula were used in a change-over design using 2 × 2 factorial arrangement. Levels of first factor (oils) were palm oil and linseed oil and levels of second factor were protected oil (calcium salts) and unprotected oil. Feeding of palm oil resulted in higher ruminal effective degradability of neutral detergent fiber (NDF) than linseed oil, and lambs fed protected oil had higher ruminal effective degradability of NDF than unprotected oil (P<0.05). Lambs fed palm oil had higher dry matter intake (DMI) and total tract digestibility of nutrients compared to those fed linseed oil. Acetate concentration in lambs fed palm oil was higher compared to linseed oil. Feeding of protected oil resulted in higher acetate concentration than unprotected oil (P<0.05). The lowest acetate concentration was found in lambs fed unprotected linseed oil (ULO) and the highest acetate concentration was observed in lambs fed unprotected and protected palm oil (UPO and PPO, respectively). Lambs fed palm oil had the highest gas and methane production compared to linseed oil. Protected oil resulted in higher gas and methane production than unprotected oil (P<0.05). Lambs fed PPO had the highest, and those fed ULO had the lowest methane production (P<0.05). Based on the results, protection of linseed oil is necessary to enhance ruminal degradation and fermentation, but this process is not necessary for palm oil.

Keywords: lambs, palm and linseed oils, ruminal fermentation, ruminal oils protection

Introduction

Lipids are added to the ruminant diets for increasing the dietary energy density at a reasonable price, also to manipulate the microbial population and ruminal fermentation processes, reduce methane production and enhance immune responses (Lourenço et al. 2010; Chamberlain and DePeters. 2017; Messana et al., 2012; Fiorentini et al., 2014; Ammah et al., 2018; Poteko et al., 2020). However, beside the positive effects, - ruminal degradability and intestinal digestibility of other - nutrients especially fiber must be protected (Hristov et al., 2013). Vegetable oils and calcium salts of fatty acids can be used in small ruminant diets (Duckett and Gillis, 2010). It is very important to assess their impacts on DMI, ruminal degradation and total tract digestion of nutrients. Palm oil and linseed oil are classified as saturated and poly unsaturated fatty acids (PUFA) sources, respectively (Pote-

ko et al., 2020; Wang et al., 2020). A significant use of linseed oil is due to its richness in alpha-linolenic acid (18:3 n-3, or cis-9, cis-12, cis-15 18:3), which result in an increase in omega-3 fatty acids in animal meat (Poteko et al., 2020). However, ruminal bio-hydrogenation of PUFA reduce the level of n-3 fatty acids flowing to the small intestine and animal meat, thus ruminal protection is needed (Lashkari et al., 2019). Calcium soap of linseed oil is a way for protection of fatty acid bio-hydrogenation (Sato et al., 2020).

There is a question whether palm oil that contains higher levels of saturated fatty acids needs to be protected compared with PUFA, such as linseed oil? And to what extent does unprotected PUFA (unprotected linseed oil) could impact on ruminal degradability of fiber, fermentation and total tract digestibility of nutrients in lambs. Our hypothesis was that unprotected palm oil should demonstrate a lower effect and unprotected linseed oil a higher effect on the ruminal degradation of fiber, fermentation and total tract digestibility of nutrients. Therefore, the objective of the present study was to evaluate the effects of the unprotected and calcium salts of palm and linseed oils on ruminal degradation and fermentation of fiber, total tract digestibility and feed intake in lambs.

Materials and methods

Animals and treatments

Four Afshari male lambs (56 kg body weight, 10 month-old) were fitted with ruminal fistula. Before the initial of experiment, the lambs were dewormed with Niclosomide (Behroodatrak Co., Arak, Iran), vaccinated against Clostridium spp. (Enterotoxaemia vaccine, Razi Institute, Karaj, Iran) and received an intramuscular injection of vitamins A, D, and E mix (Oligovit 2 mL/lamb). The experimental period lasted 48 days, and was divided into four periods of 12 days each; the first 10 days for adaptation to the diet, and 2 days for data collection. During the adaptation periods, lambs were housed in individual pens (1.0 × 1.7 m) equipped with water dispenser and water air conditioner. For sampling, the lambs were kept in metabolic cages equipped with an iron slotted floor, individual drinker, feed through, and box for collection of feces.

Four iso-energetic and iso-nitrogenous concentrate mixtures were formulated containing: 1) palmitic-rich unprotected palm oil; 2) calcium salt of palm oil; 3) linolenic-rich linseed oil; and 4) calcium salt of linseed oil (PLO). The diets were formulated based on sheep CNCPS guidelines (Cannas et al., 2003) (Table 1). Fatty acid composition of the diets is reported in Table 2.

The concentrate mix was prepared weekly to reduce lipid peroxidation and rancidity. The lipid sources were mixed with other ingredients in a vertical mixer for 10 min. The lambs were individually fed ad libitum chopped wheat straw and alfalfa and concentrates twice per day as total mixed ration at 8:00 and 16:00 h.

Table 1. Ingredient and chemical composition of experimental diets

Ingredients, g/kg of DM	
Alfalfa hay	158
Wheat straw	158
Barley grain	323
Dry corn grain	224
Soybean meal	82
Oil	39
Limestone	6
Salt	4
Vitamin premix	3
Mineral premix	3
Chemical composition, g/kg of DM	
Metabolisable energy (Mcal/kg)	2.85
Crude protein	140
Neutral detergent fiber	257
Non-fibrous carbohydrate	551
Ether extract	52

Table 2. Fatty acid composition of diets used in the experiment (based on the percentage of total fatty acids)

Fatty acids	Unprotected and protected palm oil	Unprotected and protected linseed oil
Capric (C 10:0)	2.38	1.53
Lauric (C 12:0)	4.16	0.80
Myristic (C14:0)	13.00	1.91
Palmitic (C 16:0)	50.70	2.52
Palmitioleic (C 16:1)	4.34	0.94
Stearic (C 18:0)	4.35	3.57
Oleic C18:1 (n-9)	9.10	17.80
Linoleic C18:2 (n-6)	8.60	16.21
Linolenic C18:3 (n-3)	1.08	54.18
Other fatty acids	2.38	1.36

Nutrient digestibility and degradability measurements

Apparent digestibility was determined based on the method of Block et al. (1981), using acid insoluble ash as the internal marker. Fecal samples were collected for 2 days, homogenized and a sample was taken. Feed and fecal samples were dried in an oven at 55 °C for 72 h and ground in a mill with 1 mm mesh sieve, then stored in refrigerator until chemical analysis.

On day 12 of each sampling period, ruminal fluid was collected via rumen fistula before the morning feeding. The sample was immediately filtered through double-layer of cheesecloth, and then mixed with the sterilized artificial saliva at a ratio of 1:4 under anaerobic condition. Thirty mL of the filtrated liquid were transferred to each tube containing 0.5 g dry matter of ground barley grains.

Three tubes per sample were tightly closed using stoppers fitted with syringes to collect the fermentation gas produced during incubation at 39 °C. Total cumulative gas production at 12, 24, and 48 h, and methane production at 48 h were measured.

Nylon bags (5 cm × 11 cm; pore size of 46 µm) were filled with approximately 5 g (sample size: bag surface area of 13 mg/cm^2) of the wheat straw samples ground to pass a 2 mm screen, and incubated i*n situ* according to Nocek (1988). Duplicate bags filled with wheat straw were incubated in the rumen for 0, 12, 24, 36, and 48 h. All bags were simultaneously placed in the rumen, just before the lambs were offered their first meal in the morning (i.e., 08:00 h). After retrieving from the rumen, the bags were washed three times for 5 min in a turbine washing machine and stored at -20 ºC. The same procedure was applied to two series of two bags to obtain the 0 h value. All residues were oven dried and analyzed for DM and NDF to establish degradation kinetics of wheat straw.

Chemical analysis

The nitrogen in feeds, feces and residues after rumen incubation was determined according to AOAC (Method 984.13; AOAC, 1995). The ether extract of feed and feces were determined by extraction in ether (Method 920.39; AOAC, 1995). The NDF was analyzed according to the method of Van Soest et al. (1991), using an automatic fiber analyzer (Fibertec System M, Tecator, USA). The NDF was determined using α-amylase, without sodium sulfite, without washing with acetone, and expressed without residual ash. Gross energy (GE) was determined using an adiabatic bomb calorimeter (Parr Instrument 6772®, USA).

Ruminal sampling and VFA measurements

To evaluate rumen fermentation parameters, rumen fluid samples (approximately 80 mL) were collected manually on the last day of each sampling period 4 h post AM feeding. The samples were filtered through double-layer cheesecloth, and then centrifuged at 500 × *g* for 10 min to separate the supernatant. A 20 mL sample of the supernatant was transferred to a plastic tube containing 25% (w/v) meta-phosphoric acid at a 5:1 ratio and frozen at -20 °C for volatile fatty acids (VFA) analysis based on the method of Hamada et al. (1965), and ammonia analysis following the method of Fenner (1965).

Statistical analysis

Disappearances (i.e., P) of NDF (including 0 h values) were fitted for each lamb to the exponential model of Ørskov and McDonald (1979) as: $P = a + b\,(1\text{-}e^{-ct})$. In this model, the constants "a" and "b" represent, respectively, the soluble fraction and the non-soluble but degradable component, which disappears at a constant fractional rate "c" per unit time. The effective rumen degradability (ERD) was calculated using ERD = a + bc/(c+k), estimated outflow rates (k) of 0.02, 0.05 and 0.08 /h. The data were analyzed using the generalized linear models procedure of SAS (1999) with the following statistical model: $Yijklm = \mu + C_i + R_j + oil_k + protect_l + (oil\times protection)_{kl} + e_{ijklm,}$ where *Yijk* = dependent variable, μ= overall mean, *Ci* = period effect, *Rj* = lamb number effect, oil_k = oils effect, $protect_l$ = protection effect, $(oil*protection)_{kl}$ = interaction effect, and *eijklm* = residual error, assumed normally and independently distributed. The test of Kolmogorov-Smirnov was applied to evaluate the data normality before analysis of variance. The Tukey's test was used to compare the means. Statistical differences were declared at P<0.05.

Results

NDF degradation characteristics of wheat straw are presented in Table 3. The slowly degradable fraction of wheat straw, but not the rapidly degradable fraction, was affected by oils, oil protection and their interaction (P<0.05). The slowly degradable fraction of wheat straw in lambs fed palm oil was higher than those fed linseed oil, and lambs fed protected oil had higher slowly degradable fraction of wheat straw compared to lambs fed unprotected oil (P<0.05). The effect of oils × oil protection interaction on the slowly degradable fraction is reported in Table 7. The highest slowly degradable fraction of wheat straw was observed in lambs fed PPO (P<0.05), and the lowest one in lambs fed ULO (P<0.05). Degradation rate and ruminal effective degradability of NDF were affected by oils and oil protection (P<0.05), but not by their interaction. Feeding of palm oil resulted in higher degradation rate and ruminal effective degradability of NDF than linseed oil (P<0.05). Lambs fed diet containing protected oil had higher degradation rate and ruminal effective degradability of NDF compared to lambs fed unprotected oil (P<0.05).

The DMI and total tract digestibility of experimental diets are presented in Table 4. The DMI was affected by oils and oil protection, but not by their interaction (P<0.05). Digestibility of OM and GE was affected by oils (P<0.05), but not by oil protection and the interaction. Digestibility of CP was similar among treatments, but digestibility of NDF was affected by main factors and their interaction. Lambs fed palm oil had higher DMI and digestibility of OM, NDF and GE than those fed linseed oil (P<0.05). Feeding protected oil resulted in higher NDF digestibility compared with unprotected oil (P<0.05). The interaction effect of oil × oil protection on NDF digestibility is presented in Table 7. Lambs fed UPO, PPO and PLO had the same digestibility, but ULO had the lowest total tract OM, NDF, and GE digestibility (P<0.05).

The effects of oils and oil protection and their interaction on ruminal ammonia level and VFA concentration are presented in Table 5, and the effect of oils × oil protection interaction on acetate concentration is reported in Table 7. Ruminal ammonia and butyrate -

concentrations were similar among all traetments. Propionate and isobutyrate concentrations, and acetate to propionate ratio were affected by oils, but not by oil protection and oils × oil protection interaction. Acetate concentration was affected by oils, oil protection and oils × oil protection interaction. Lambs fed linseed oil had the higher propionate and isobutyrate concentration than palm oil (P<0.05). Acetate concentration in lambs fed palm oil was higher compared to linseed oil (P<0.05). Feeding protected oil resulted in higher acetate concentration than unprotected oil (P<0.05). The lowest acetate concentration was found in lambs fed ULO and the highest acetate concentration was found in lambs fed UPO and PPO (P<0.05; Table 7).

Table 3. Neutral detergent fiber degradation characteristics of wheat straw in the rumen of lambs fed unprotected and protected palm and linseed oils

	Oils sources		Oil protection			P Value[2]		
Parameters[1]	Palm	Linseed	Unprotection	Protection	SEM	O	P	I
a fraction (%)	6.75	6.85	6.80	6.80	0.192	0.613	0.990	0.142
b fraction (%)	42.35[a]	37.40[b]	36.65[b]	43.10[a]	0.914	0.002	0.001	0.016
c (% / h)	6.10[a]	5.75[b]	5.70[b]	6.15[a]	0.079	0.008	0.001	0.539
ED (%)	29.95[a]	26.85[b]	26.31[b]	30.50[a]	0.960	0.007	0.001	0.274

[1] a fraction, rapidly soluble fraction; b fraction: slowly degradable fraction (b, %), c: degradadion rate; ED: ruminal effective degradation

[2] P value for O: oils, P: oil protection and I: interaction (oils × oil protection)

[a,b]: Within rows in each section, mean values with common letter(s) are not different (P>0.05).

Table 4. Dry matter intake and total tract digestibility coefficients in lambs fed unprotected and protected palm and linseed oils

	Oil sources		Oil protection			P Value[1]		
Parameters	Palm	Linseed	Unprotection	Protection	SEM	O	P	I
Dry matter intake (g/lamb/day)								
	1721[a]	1698[b]	1699[b]	1719[a]	7.56	0.010	0.021	0.310
Apparent digestibility coefficient (%)[2]								
OM	70.51[a]	68.70[b]	69.45	9.75	0.600	0.011	0.626	0.004
CP	80.61	80.75	80.65	80.55	0.610	0.990	0.872	0.271
NDF	47.15[a]	44.35[b]	44.95[b]	46.55[a]	0.424	0.001	0.002	0.002
GE	71.10[a]	70.75[b]	71.12	71.75	0.455	0.011	0.178	0.003

[1] P value for O: oils, P: oil protection and I: interaction (oils × oil protection)

[2] OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, GE: gross energy

[a,b]: Within rows in each section, mean values with common letter(s) are not different (P>0.05).

Table 5. Ammonia and volatile fatty acids levels in the rumen of lambs fed unprotected and protected palm and linseed oils

	Oil sources		Oil protection			P Value[1]		
Parameters	Palm	Linseed	Unprotection	Protection	SEM	O	P	I
NH3 (mg/dl)	15.43	15.7	14.96	16.15	0.756	0.761	0.146	0.124
Volatile fatty acids (molar %)								
Acetate	48.65[a]	41.10[b]	44.32[b]	45.43[a]	0.235	0.001	0.008	0.001
Propionate	33.51[b]	40.25[a]	37.05	36.71	0.181	0.001	0.077	0.787
Butyrate	17.15	17.10	17.33	16.95	0.649	0.939	0.559	0.132
Isobutyrate	0.60[b]	0.85[a]	0.72	0.75	0.069	0.003	0.486	0.055
A:P ratio[2]	1.44[a]	1.03[b]	1.24	1.23	0.033	0.001	0.883	0.659

[1] P value for O: oils, P: oil protection and I: interaction (oils × oil protection)

[2] Acetate to propionate ratio

[a,b]: Within rows in each section, mean values with common letter(s) are not different (P>0.05).

Table 6. The main factors and interaction effects of oils and protection on *in vitro* gas and methane production

	Oils sources		Oil protection			P Value[1]		
Parameters	Palm	Linseed	Unprotection	Protection	SEM	O	P	I
Gas production (mL/0.5 g DM)								
12 h	99.9[a]	90.5[b]	93.3[b]	97.1[a]	0.97	0.001	0.002	0.910
24 h	132.5[a]	112.0[b]	116.3[b]	128.3[a]	1.51	0.001	0.001	0.155
48 h	142.1[a]	118.6[b]	124.2[b]	136.7[a]	2.87	0.001	0.001	0.772
Methane production[2]	12.2[a]	6.1[b]	7.9[b]	10.3[a]	1.27	0.001	0.001	0.002

[1] P value for O: oils, P: oil protection and I: interaction (oils × oil protection)

[2] Total methane production after 48 h incubation (mL/0.5 g DM)

[a,b]: Within rows in each section, mean values with common letter(s) are not different (P>0.05).

The main and interaction effects of oils and oil protection on *in vitro* gas and methane production are presented in Table 6. Gas and methane production were affected by the main factors, but not by their interaction, except for methane production, which was affected by the interaction. Lambs fed palm oil had the highest gas -

and methane production than linseed oil (P<0.05). Feeding protected oil resulted in higher gas and methane production than unprotected oil (P<0.05). Lambs fed PPO had the highest, and those fed ULO had the lowest methane production (P<0.05). The ULO and PLO had lower methane production, as compared with UPO and PPO (P<0.05; Table 7).

Table 7. Interaction effect of oils and oil protection on total tract digestibility, ruminal degradability, acetate concentration and methane production

Parameters	Unprotected Palm oil (UPO)	Protected Palm oil (PPO)	Unprotected Linseed oil (ULO)	Protected linseed oil (PLO)	SEM	P value
Total tract digestibility (%)						
Organic matter	71.4[a]	69.6[a]	67.5[b]	69.9[a]	0.67	0.005
Neutral detergent fiber	47.5[a]	46.8[a]	42.4[b]	46.4[a]	0.44	0.001
Gross energy	72.2[a]	71.6[a]	69.8[b]	71.9[a]	0.45	0.003
In situ degradability (%)						
a fraction (%)1	40.4[b]	44.3[a]	32.9[c]	41.9[ab]	0.91	0.001
Volatile fatty acids (molar %)						
Acetate (molar %)	48.8[a]	48.7[a]	40.2[c]	42.4[b]	0.10	0.001
Total methane production after 48 h incubation (mL/0.5 g DM)						
Methane production	11.2[b]	13.1[a]	4.6[d]	7.5[c]	0.12	0.001

[a,-d]: Within rows, mean values with common letter(s) are not different (P>0.05).

Discussion

We evaluated the main and interaction effects of unprotected (raw) and protected (calcium salts) palm and linseed oils on total tract digestion, ruminal degradation and fermentation in sheep. Many studies reported that lipid inclusion in the ruminant diet had either positive or negative effects, especially on nutrient degradability and digestibility (Messana et al., 2012; Fiorentini et al., 2014; Ammah et al., 2018; Poteko et al., 2020). Such inconsistencies could be due to several factors, such as the level and source of dietary lipids and the nature of the basal diet, which in turn influences the effects of lipids on ruminal microbes (Messana et al., 2012; Fiorentini et al., 2014; Benchaar et al., 2015). In the present study, protected oil resulted in higher ruminal degradability of NDF than unprotected oil. Physical and chemical mechanisms have been explained for these modifications (Jacob et al., 2012; Benchaar et al., 2015; Lamp et al., 2018; Francisco et al., 2019). The PUFA are toxic to protozoa, fibrolytic bacteria, and methanogenic bacteria (Francisco et al., 2019; Ma et al., 2022). Moreover, including oils in the diet can coat feed particles (Lamp et al., 2018), which limits the microorganism's colonization on particles. Particle colonization is a necessary step for the hydrolysis of plant components by bacterial enzymes (Ma et al., 2022). These two mechanisms decrease the fibrous digestion rate and amount.

The inclusion of unprotected linseed oil to the diet resulted in lower effective ruminal degradability of NDF than palm oil. Linseed oil contains poly-unsaturated fatty acids, which are toxic substances to ruminal fibrolytic bacteria (Jacob et al., 2012; Benchaar et al., 2015). Dietary inclusion of protected palm oil had a lower negative effect on fiber degradation in the rumen, but unprotected palm oil reduced the ruminal degradability of fiber. Unprotected fatty acids (saturated or unsaturated) could reduce calcium and magnesium ions levels in the ruminal fluid and result in the reduction of cellulolytic activity and, finally reduce the ruminal degradation of fiber. Fibrolytic enzymes need calcium and magnesium ions to attach to fiber particles (Silva and Dehority, 2009) for the degradation of cellulosic compounds.

In the present study, DMI was higher in lambs-fed palm oil than lambs-fed linseed oil. In contrast to the present study, Vandoni et al. (2010) found an increase in dry matter intake after including unprotected fat in the diets of lambs compared with animals given calcium salt of fat. Conflicting data on palatability exist. There were reports of no change in DMI (Preziuso et al., 1999), decreased (Fiorentini et al., 2014; Behan et al., 2019), or increased DMI (Salinas et al., 2006) with protected lipids supplementation compared with unprotected lipids. Reduction in the fiber digestibility in lambs fed linseed oil may be accompanied by a reduction in DMI as fiber accumulates as the mat above the ruminal fluid and decrease the fiber passage rate. In addition, the reduction of fiber degradability by the inclusion of linseed oil, a decrease in the other nutrients digestibilities occurred. These events compromised the dietary energy value, the greater ruminal retention time of fiber, lower passage rate and finally, limited the dry matter intake.

According to Oliveira et al. (2020), DMI is highly related to dietary NDF level and degradation rate, fibre quality and particle size. Linseed oil reduced the ruminal NDF degradation. Fiber fermentation and passage rate in the rumen of lambs receiving linseed oil are slower than other dietary treatments, leading to rumen fill, higher residence time and lower dry matter intake. According to Weld and Armentano (2017), lower NDF degradability may be due to a higher presence of unsaturated fatty acids that affects the permeability of the bacterial membranes. In the present study, including protected linseed oil increased dry matter intake and total tract digestibility of nutrients compared to unprotec-

ted linseed oil. This finding suggests that the calcium salt of linseed oil could decrease the fiber accumulation and the negative effects of linseed oil (unprotected) in the rumen.

Our finding is accordance with the finding of Fiorentini et al. (2015), who reported that protein digestibility was not affected by the addition of saturated tallow or unsaturated soybean oil in sheep diets, whereas the apparent fiber digestibility was significantly lowered with the inclusion of linseed oil. The effects of oils and oil protection and their interaction on total tract digestibility of OM, NDF and GE were significantly different. This finding is consistent with studies conducted by Naik et al. (2009), who found that feeding rumen-protected lipids to buffaloes had no influence on the digestibility of DM, OM, CP, total carbohydrates and NDF. The current findings are inconsistent with the findings of Beauchemin et al. (2007) who reported that dietary lipid sources had no effect on DM, GE and NDF digestibility. This difference could be due to the level of protected fat in the diet (90 g/kg of total diet vs. 50 g/kg in the present study).

Acetate concentration in lambs-fed palm oil was higher than lambs receiving linseed oil. This is related to the higher ruminal degradability of NDF in the rumen of lambs fed with the diet containing palm oil, compared to those fed linseed oil (Table 3). The ULO reduced acetate level compared to PLO (Table 7). Unsaturated fatty acids in ULO had toxic effects on Gram-positive bacteria (Maia et al., 2010), which reduce the fiber degradation by microorganisms (Lourenço et al., 2010). In the present study, the NDF degradability, and thus acetate production, decreased in lambs fed with ULO.

The similar rumen fermentation parameters, including ammonia production and molar proportions of VFA, are consistent with a study reporting that rumen ammonia levels were not affected adversely by the supplementation of calcium soaps up to five percent of the DM in lactating cows (He et al., 2018).

The concentrations of acetate, propionate and acetate to propionate ratio were different in the present study throughout the treatments, which is inconsistent with the report of Behan et al. (2019), who reported that neither the source nor the level of rumen protected lipids influenced VFA level and acetate-to-propionate ratio in steers fed protected lipids and hydrogenated animal fats. In the present study, no differences were found on VFA concentrations for protection levels. Similarly, no differences were observed in ruminal VFA concentration by feeding three different types of rumen protected and unprotected lipids (Demirel et al., 2004).

In the present study, dietary inclusion of linseed oil reduced and protection induced significantly gas and methane production. Many reports shown that dietary inclusion of linseed oil, and to a lower extent palm oil, decreased the ruminal gas and methane production (Martin et al. 2008; Fiorentini et al., 2014; Benchaar et al., 2015; Sato et al., 2020). A meta-analysis conducted by Martin et al. (2008) demonstrated that methane production decreased by 4.8% for each 1% inclusion of linseed oil. Unsaturated fatty acids in unprotected oils may efficiently suppress the activity of methanogenic bacteria (Poteko et al., 2020). As seen in Table 7, calcium salts of oil produce more gas and methane production, as compared with unprotected oils. Dietary inclusion of calcium salts of palm oil and linseed oil increased methane production *in vitro* by 15% and 61% compared to unprotected oils, respectively. This means unsaturated fatty acids could reduce population and activity of fibrolytic bacteria as seen in ruminal acetate level and also methanogenic bacteria.

Conclusion

This study revealed that ruminal protection of linseed oil is necessary to enhance ruminal degradation and fermentation and total tract digestibility. Although, unprotected palm oil had almost no or lower negative effect on ruminal degradation and fermentation, it is suggested to be used as its calcium salt.

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Conflict of interest

There is no conflict of interest.

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