Effects of dietary oil supplementation on the rumen ciliate protozoa and fiber-degrading bacteria in goats

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Abstract

Oil supplementation in the ruminant diet has been proven to reduce the rumen protozoa population and maintain their low amount during supplementation. Majority of oil that contains medium chain fatty acids are also known to reduce cellulolytic bacteria. This experiment was conducted to study the effect of different types of oil supplementation on the protozoa and fiber-degrading bacteria population in goats. Sixteen male goats equipped with rumen cannula were assigned to four experimental dietary treatments. The first group acted as the control group (CON) and received the basal diet. The second group (OL) received the basal diet with the supplementation of olive oil, whereas the third group (SO) and fourth group (PL) received the basal diet supplemented with sunflower and palm olein oils, respectively. The rumen content of each animal was collected for pH measurement as well as enumeration of the rumen protozoa and fiber-degrading bacteria. The rumen pH level was affected by sampling days ($P<0.05$). The total protozoa counts were higher in the CON compared to the other treatments, which the OL group had the lowest rumen protozoa counts and were significantly affected ($P<0.05$) by the diet and day of sampling. Treatment groups did not affect *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* populations, but there were significantly affected ($P<0.05$) by day of sampling and the interaction of treatment × day. In conclusion, dietary oils supplementation affects the rumen protozoa population, but the effects on bacteria population are not extensive.

Keywords: Rumen protozoa, fiber degrading bacteria, olive oil, palm olein oil, sunflower oil

Introduction

Protozoa are one of the natural microbial populations, besides bacteria and fungi that are found in the rumen of ruminants (McSweeney *et al*., 2007). The rumen protozoa can be divided into two groups, namely holotrich and entodiniomorphid ciliates. These two groups of the ciliate protozoa occupy different metabolic niches in response to certain dietary condition. Holotrichs consume soluble carbohydrates and entodiniomorphid ciliates utilize and ferment particulate materials (Williams & Coleman, 2012). Thus, the number of rumen protozoa is diet-dependent. Animal nutritionists have been working on improving the nutrient utilization of ruminants by manipulating the rumen microbial ecosystem to enhance fibrous feed digestibility, reduce methane emission, and reduce nitrogen excretion (Patra *et al.*, 2006; Pilajun & Wanapat, 2011). Feed additives have been shown to have direct impact on the population of rumen protozoa. Addition of ionophores such as monensin (Poos *et al.*, 1979; Hino &

Russel, 1987), plant secondary compounds such as tannins and saponin (Poungchompu *et al.*, 2009; Liu *et al.*, 2011), were found to inhibit the rumen protozoa population. Apart from the feed additives listed above, it has been discovered that dietary lipids can also act as an effective modifier of ruminal fermentation but they are found to be toxic to the protozoa (Machmüller *et al.*, 1998). Inclusion of dietary lipid in ruminant diet may improve the efficiency of protein utilization by reducing the intraruminal recycling of bacterial protein (Hristov & Jouany, 2005). However, the inhibitory effects on the protozoa population are influenced by the degree of fatty acid unsaturation. Some researchers have claimed that higher unsaturation caused more inhibitory effects on the ruminal protozoa population (Jenkins, 1993; Roy *et al.*, 2017). Supplementation of sunflower oil in the ruminant diet was found to result in a massive reduction of the protozoa population and it is believed to be due to the content of linoleic acid (C18:2) which inhibits the number of rumen ciliate protozoa (Ivan *et al.*, 2001). Oil supplementation on rumen microbial population is more multifaceted. The effects depend more on fatty acid composition and the degree of saturation in the oils (Jenkins, 1993). To our knowledge, there is not much information available regarding the effect of diets supplemented with olive oil that contains oleic acid (C18:1) and palm olein oil that contains a combination of linoleic acid and palmitic acid (C16:0) on the rumen ciliate protozoa and fiber-degrading bacteria in rumen. Thus, the aim of this study was to investigate the effect of olive oil, palm olein oil or sunflower oil supplementation on the protozoa and fiber-degrading bacteria population in goats.

Materials and Methods

Animals and diets

Sixteen local crossed male goats equipped with rumen cannula and aged between 20–24 mo (mean body weight of 28.32 ± 1.85 kg) were randomly assigned according to a completely randomized design to four dietary treatment groups, as follows: CON (basal diet receiving no oil supplement); OL (basal diet + 6 % olive oil); SO (basal diet + 6 % sunflower oil); and PL (basal diet + 6 % palm olein oil). The diets were formulated to have approximately equal amount of crude protein (CP) and energy content (NRC, 2007). The ingredients of the diets are presented in Table 1. Proximate analysis consisting of dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) for all treatment diets was analyzed using AOAC (2012) procedure and Van Soest *et al.* (1991) method. The diets were offered *ad libitum* to the goats at 09:00 h daily. The goats were kept individually in separate pens and had free access to water. The proximate analysis of the diets is presented in Table 2.

Table 1. Ingredients of the treatment diets with different dietary oil inclusions

Ingredient (as fed)	Treatments			
	CON	OL	PL	SO
Rice straw (%)	30.8	25.8	25.8	25.8
Barley grain (%)	35.0	35.0	35.0	35.0
Soybean meal (%)	30.0	30.0	30.0	30.0
Molasses (%)	2.0	1.0	1.0	1.0
Vitamin mineral-mix (%)	0.5	0.5	0.5	0.5
Limestone (%)	1.3	1.3	1.3	1.3
Sodium sulphate (%)	0.4	0.4	0.4	0.4
Olive oil (%)	-	6.0	-	-
Sunflower oil (%)	-	-	-	6.0
Palm oil (%)	-	-	6.0	-

Note: CON=basal diet; OL=basal diet + olive oil; PL=basal diet + palm olein oil; SO=basal diet + sunflower oil.

Table 2. Chemical composition of the treatment diets with different dietary oil inclusions

Chemical analysis (DM %)	Treatments			
	CON	OL	SO	PL
DM	76.17	76.02	78.73	78.27
OM	93.60	93.34	94.96	94.61
EE	1.86	4.56	4.74	4.70
CP	15.76	15.48	16.00	15.9
NDF	63.53	58.76	51.27	58.54
ADF	17.04	18.26	21.41	20.66

Note: CON=basal diet; OL=basal diet + olive oil; PL=basal diet + palm olein oil; SO=basal diet + sunflower oil; DM=dry matter; OM=organic matter; CP=crude protein; EE=ether extract; NDF=neutral detergent fiber; ADF=acid detergent fiber

Sampling and analysis

The study started with 28 d of adjustment period followed by 30 d of experimental period. At the end of the adjustment period, on day 28, the animals were divided into a group of four goats and given the respective experimental diets. Prior to this, the rumen samples were taken on days 27 and 28 of the adjustment period which was considered as the initial day or at 0 day of experimental period. The rumen fluids collected on days 27 and 28 of the adjustment period were pooled and kept at room temperature and subjected to the protozoa count analysis. Similarly, the rumen samples were also collected on days 2, 4, 6, 12, 18, 24, and 30 of the experimental period. The pH of the rumen content was measured immediately using a portable pH meter (Eco Testr pH 1, Eutech Instrument Pte Ltd, SGP). Five ml of the filtrates were preserved in 5 ml of 10 % formal saline and staining was done using brilliant green dye as described by Dehority (1993). The holotrich and entodiniomorphid ciliates were counted using a Neubauer Improved Bright-Line counting cell chamber (0.1 mm depth; Hausser Scientific, Horsham, PA, USA).

Each sample was counted twice, and if the coefficient of the variation was greater than 10%, the counts were repeated. The total protozoa count was calculated as a sum of holotrich and entodiniomorphid ciliates counts.

DNA from rumen content samples collected on day 0, 12 and 30 were extracted using QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the guidelines provided by the manufacturer. Real-time PCR was used to determine the population of prominent fiber-degrading bacteria: *F. succinogens*, *R. albus* and *R. flavefaciens*. Species-specific PCR primers that were used to amplify partial 16S rDNA regions are presented in Table 3. Real-time PCR amplification and detection were performed using CFX 96 system (Bio-Rad, Hercules, CA, USA). The amplification reaction was conducted in a final volume of 25 µl which contained 12.5 µl Maxima SYBR Green qPCR Master Mix, 8.5 µl RNAse free distilled water, 2 µl of DNA elution and 1 µl of each species-specific PCR primer. PCR conditions of all species were as follows: 15 s at 95°C for denaturing, 30 s at annealing temperature, and 20 s at 72°C for an extension for 39 cycles. The standards used in this study were prepared according to the protocol described by Navidshad *et al*. (2012)

Table 3. The PCR primer used for quantification of fiber-degrading bacteria in goat fed with different dietary oils

Fiber-degrading bacteria	Primer		Amplicon (base pairs)	Ref
	Forward	Reverse		
F. succinogenes	5'-GTTCGGAATTACTGGG CGTAAA-3'	5'-CGCCTGCCCCTGA ACTATC-3'	121	[1, 2]
R. albus	5'-CCC TAA AAG CAG TCT TAG TTC G-3'	5'-CCT CCT TGC GGT TAG AAC A-3'	175	[3]
R. flavefaciens	5'-CGAACGGAGATAATT TGAGTTTACTTAGG-3'	5'-CGGTCTCTGTATGTTA TGAGGTATTACC-3'	132	[1, 2]

Ref = references
1, Samsudin *et al*. (2014); 2, Denman & McSweeney (2006); 3, Koike & Kobayashi (2001)
[#]Published in Ibrahim *et al.*, 2016.

Statistical analysis

Data analysis was performed using the general linear model (GLM) procedure of SAS (2003). It was used to analyze the data of rumen pH, protozoa count and fiber-degrading bacteria population affected by the dietary treatments, days of sampling, and treatments × day of sampling interaction in the model. Duncan Multiple Range Test was used to further compare means at $P<0.05$.

Results and Discussion

The mean ruminal pH as shown in Table 4 ranged between 6.26 (PL) to 6.80 (OL) and was affected by the day of sampling ($P<0.05$). Different treatment diets had no significant effect on the ruminal pH. However, the OL fed group had slightly higher rumen pH value pattern than the other groups (Figure 1).

Table 4. Effect of basal diets and dietary oil supplements on the rumen ciliate protozoal and fiber degrading bacteria populations

Parameter	Treatment				SEM	P value		
	CON	OL	SO	PL		Treatment	Day	Treatment × Day
pH	6.29	6.80	6.33	6.26	0.09	0.58	0.00	0.85
Protozoa count 10^4 mL^{-1}								
Total protozoa*	24.50^a	10.68^b	12.86^b	11.09^b	0.56	0.00	0.00	0.73
Entodiniomorphids	19.45^a	8.64^b	10.44^b	8.44^b	0.46	0.01	0.00	0.76
Holotrichs	5.05^a	2.04^b	2.42^b	2.65^b	0.09	0.00	0.00	0.00
Fiber-degrading bacteria#								
F. succinogenes ×10^4/ml)	6.04	8.60	4.42	4.730	5.62	0.06	0.03	0.01
R. albus (×10^5/ml)	1.91	1.33	4.39	11.50	2.22	0.78	0.02	0.01
R. flavefaciens (×10^8/ml)	2.24	7.18	3.33	6.05	1.29	0.31	0.01	0.00

Note:
CON=basal diet; OL=basal diet + olive oil; PL=basal diet + palm olein oil; SO=basal diet + sunflower oil;
*Total protozoa; sum of holotrichs and entodiniomorhids.
#Published in Ibrahim *et al.*, 2016.
Values are expressed in mean (n = 4).

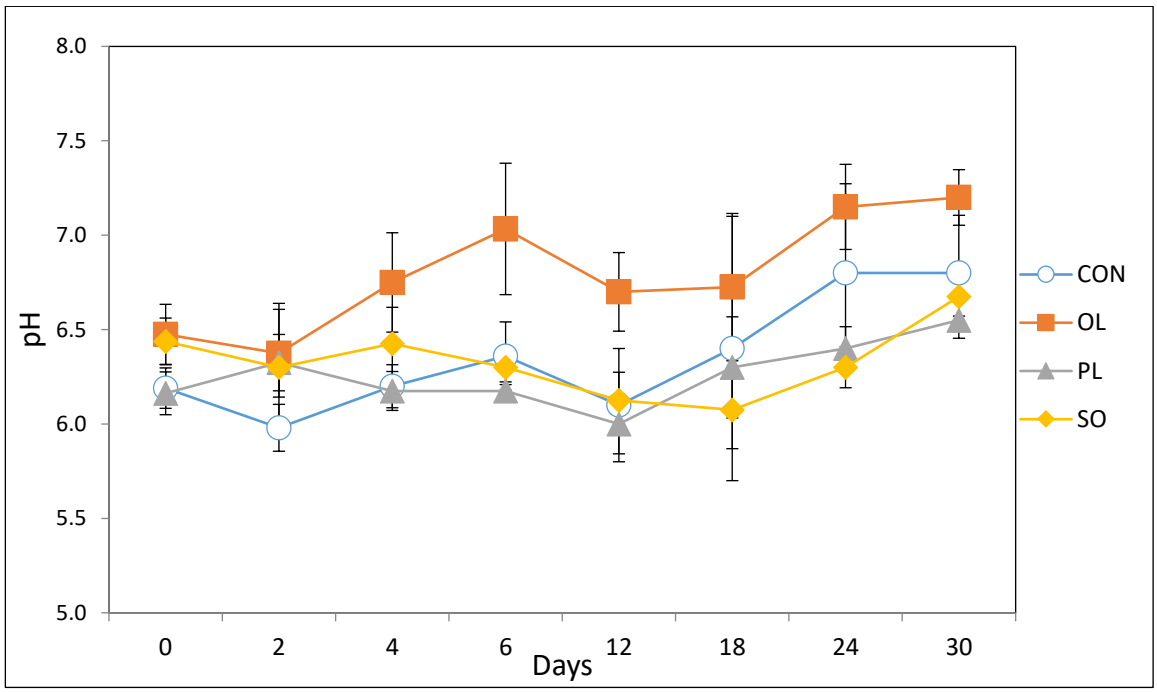

Figure 1. Effects on pH value (means ± SE) in rumen fluid of goats supplemented with dietary oils at different days. Vertical bars are standard errors. (CON=basal diet; OL=basal diet + olive oil; PL=basal diet + palm olein oil; SO=basal diet + sunflower oil).

The effect of dietary oil supplementation on the rumen pH did not differ significantly among the treatments. However, there were significant differences in ruminal pH over days of sampling. This result suggests that the rumen microbial population is able to adapt to the diet given, regardless of the addition and difference in composition of the dietary oil supplemented (Penner *et al.,* 2006).

Although there was no statistically significant difference between the treatments, supplementation of olive oil tended to increase the rumen pH compared to the other treatment groups, where the effect appeared to increase on day 6. However, the values were within normal range and did not alter the rumen cellulolysis processes of fiber and protein digestion (de Veth & Kolver, 2001; Wales *et al.,* 2004). Nevertheless, the pH values in the other dietary treatments were also within the normal range of ruminal pH value.

The findings of the present study are consistent with Anantasook *et al.* (2013) who reported that supplementation of palm oil did not give negative effects on the ruminal pH in dairy cows. Moreover, Pilajun *et al.* (2010) showed that pH values in cattle supplemented with sunflower oil were between 6.6–6.8, depending on its combination level with the coconut oil.

The mean number of protozoa count was significantly higher ($P<0.05$) in CON group compared to the other treatment groups which the OL group had the lowest rumen protozoa population. The total protozoa count was significantly affected ($P<0.05$) by the dietary treatments and day of sampling. However, there was no significant interaction between dietary treatment and day of sampling. The total protozoa count and individual number of holotrich and entodiniomorphid ciliates are shown in Table 4.

The protozoa number in rumen contents is influenced by the feeding practices. Although protozoa form the fundamental part of the microbial population in the rumen, their benefit to the ruminant is still controversial (Ishler *et al.,* 1996). The rumen ciliate protozoa population was found to be at low level when the animals were fed with dietary oil (Tesfa, 1993; Yanez Ruiz *et al.,* 2004; Wanapat & Khampa, 2006). Several other studies (Cieślak *et al.,* 2013; Patra and Yu, 2013; Benchaar *et. al.,* 2015) also demonstrated similar result to this study which shows that the number of protozoa population in the rumen tends to decrease when the animals are fed with different sources of dietary oils.

The decrease in total protozoa count in the rumen fluid was probably due to the toxic effect of the supplemented oils in the diet. It has been postulated that the unsaturated fatty acids are toxic to the rumen ciliate protozoa (Newbold & Chamberlain, 1998). However, the concentration of the dietary lipid must be adequate to particularly affect the rumen protozoa population because of the ability of protozoa to absorb and metabolize lipid unlimitedly leading to the swelling and rupture of their cells in the high concentration of dietary lipid (Williams & Coleman, 2012; Girard & Hawke, 1978). Previous studies have shown contradictory effects of different concentration of dietary lipid supplementation. Anantasook *et al.* (2013) reported at 2% dietary lipid supplementation decreased protozoa population. On the other hand, Benchaar *et al.* (2012) did not observe similar result even at 4% dietary lipid supplementation. It has also been observed by Ivan *et al.* (2001) and Yanez Ruiz *et al.* (2004) that holotrichs were most vulnerable to the toxic effect of the oils rich in fatty acids which contributed to the reduced number of the total protozoa. Apart from that, the duration of dietary oil supplementation to the animal also had an effect in reducing the rumen protozoa population (Ivan *et al.,* 2001).

The effect of oil supplementation on fiber-degrading bacteria population is presented in Table 4. There were no significant differences (P>0.05) observed in the *F. succinogenes, R. albus and R. flavefaciens* populations as affected by treatment diet. However, significant differences (P<0.05) were observed for all fiber-degrading bacteria populations which were influenced by day of sampling and the interaction of treatment × day.

Different responses of oil supplementation on fiber-degrading rumen bacteria were shown in this study. High number of *F. succinogenes* recorded in OL, *R. flavefaciens* in all of the supplemented diets, and significant increase of *R. albus* in the PL and SO groups compared to CON. These results may suggest that oil supplementation did not directly affect the fiber-degrading bacteria population in the rumen. Similarly, Ivan *et al.* (2013) observed an increased population of *R. albus* and *R. flavefaciens* with oil supplementation. In addition, Yabuuchi *et al.* (2007) reported that the negative effects towards fiber-degrading bacteria were neglected in the case of high grain feed diet. Besides types of dietary oils supplemented, Wanapat *et al.* (2011) and Marín *et al.* (2012) suggested that other factors such as method of administration and level of inclusion in the diet might also influence the effects on rumen.

Conclusion

In conclusion, the above results suggest that the supplementation of goat diet with dietary oils can positively reduce the rumen protozoa population. In addition, oil supplementation did not have any adverse effects on fiber-degrading bacteria populations, thus did not compromise the rumen fiber digestion activity.

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References

Anantasook, N., Wanapat, M., Cherdthong, A. and Gunun. P. 2013. Effect of plants containing secondary compounds with palm oil on feed intake, digestibility, microbial protein synthesis and microbial population in dairy cows. Asian Austral. J. Anim. Sci. 26(6): 820-826.

AOAC. 2012. Association of Official Analytical Chemistry. Official methods of analysis. AOAC International. Gaithersburg MA, USA.

Benchaar, C., Romero-Pérez, G.A., Chouinard, P.Y, Hassanat, F, Eugene, M, Petit, H.V. and Côrtes, C. 2012. Supplementation of increasing amounts of linseed oil to dairy cows fed total mixed rations: Effects on digestion, ruminal fermentation characteristics, protozoal populations, and milk fatty acid composition. J. Dairy Sci. 95(8): 4578-4590.

Benchaar, C., Hassanat, F., Martineau, R. and Gervais R. 2015. Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. J. Dairy Sci. 98(11):7993-8008.

Cieślak, A., Váradyová, Z., Kišidayová, S., Jalč, D. and Szumacher-Strabel, M. 2013. Effect of diets with fruit oils supplements on rumen fermentation parameters, fatty acid composition and methane production in vitro. J. Anim. Feed Sci. 22(1): 26-34.

Dehority, B.A. 1993. Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa. CRC Press, USA.

Denman, S.E. and McSweeney, C.S. 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiol Ecol. 58(3): 572-582.

de Veth, M.J. and Kolver, E.S. 2001. Diurnal variation in pH reduces digestion and synthesis of microbial protein when pasture is fermented in continuous culture. J. Dairy Sci. 84 (9): 2066–2072.

Girard, V. and Hawke, J.C. 1978. The role of holotrichs in the metabolism of dietary linoleic acid in the rumen. Biochem Biophys Acta 528: 17-27.

Hino, T. and Russell, J.B. 1987. Relative contributions of ruminal bacteria and protozoa to the degradation of protein in vitro. J. Anim. Sci. 64: 261-270.

Hristov, A.N. and Jouany, J.P. 2005. Factors affecting the efficiency of nitrogen utilization in the rumen. In: A.N. Hristov, & E. Pfeffer (Eds). Nitrogen and Phosphorus Nutrition of Cattle and Environment. CAB International, Wallingford, p. 117–166.

Hristov, A.N., Ivan, M. and McAllister, T.A. 2004. In vitro effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high-concentrate, barley-based diet. J. Anim. Sci. 82(9): 2693-2704.

Ibrahim, N.A., Alimon, A.R, Yaakub, H., Abdullah, N. and Samsudin, A.A. 2016. Effects of dietary oil supplementation with different fatty acid profiles on rumen fiber degrading bacteria population in goats. In: Proc. International Seminar on Livestock Production and Veterinary Technology. 271-275.

Ishler, V.A., Heinrichs, A.J. and Varga, G.B. 1996. From Feed to Milk: Understanding Rumen Function (Vol. 422). Pennsylvania State University. University Park, PA.

Ivan, M., Mir, P.S., Koenig, K.M., Rode, L.M, Neill, L., Entz, T. and Mir, Z. 2001. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Rum. Res. 41: 215-227. https://doi.org/10.1016/S0921-4488(01)00220-6

Ivan, M., Petit, H.V., Chiquette, J. and Wright, A.D. 2013. Rumen fermentation and microbial population in lactating dairy cows receiving diets containing oilseeds rich in C-18 fatty acids. Brit J Nutr. 109(7): 1211-1218.

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

Koike, S. and Kobayashi, Y. 2001. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus and Ruminococcus flavefaciens*. FEMS Microbiol Lett. 204(2): 361-366.

Liu, H., Vaddella, V. and Zhou, D. 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. J. Dairy Sci. 94(12): 6069-6077.