doi: 10.17221/9/2017-CJAS

Supplementation of Rapeseed and Linseed Oils to Sheep Rations: Effects on Ruminal Fermentation Characteristics and Protozoal Populations

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ABSTRACT

Majewska M.P., Miltko R., Bełżecki G., Skomiał J., Kowalik B. (2017): **Supplementation of rapeseed and linseed oils to sheep rations: effects on ruminal fermentation characteristics and protozoal populations**. Czech J. Anim. Sci., 62, 527–538.

The study was performed on six sheep fitted with a cannula in the rumen and re-entrant cannula in the duodenum; divided into three groups, two sheep in each. The animals were fed meadow hay and the concentrate alone or the same diet supplemented with rapeseed or linseed oils at a dose of 5% of the basal diet. Ruminal degradation of protein and acid detergent fibre were lower when sheep were fed rapeseed compared to linseed oil ($P < 0.05$). The addition of oils to diets caused increased ruminal degradation of fat ($P < 0.01$). The density of protozoa in the rumen at 2 or 4 h after feeding was lower than before feeding in each experimental group. The inclusion of rapeseed oil in the diet decreased the total number of ciliates and *Entodinium* spp. compared with control and animals fed linseed oil ($P < 0.01$). Before feeding, the concentration of *Diplodinium* and *Ophryoscolex* spp. were lower in sheep fed rapeseed oil compared to control ($P < 0.05$), and the number of *Dasytricha* species decreased 2 h after feeding linseed oil compared to animals fed rapeseed oil ($P < 0.05$). Each of the oil supplements decreased the bacterial mass in the rumen compared with control ($P < 0.01$). The addition of rapeseed oil to the diet decreased total volatile fatty acid and acetate concentrations in the rumen in comparison to control and sheep receiving linseed oil ($P < 0.01$). In both diets, the estimated emission of methane and carbon dioxide ($P < 0.01$) increased 2 and 4 h after feeding compared to that at 0 h. The oleic acid more strongly reduced protozoa and digestive processes in the rumen than linolenic acid. Nevertheless, the quantity of oils added was still too low to induce detectable changes in methane formation in the rumen.

Keywords: ruminant; vegetable oils; fermentation pattern; protozoa; ruminal degradability

Supplementation of diets with fat for domestic ruminants may profoundly affect feed intake and nutrient digestibility, thereby changing the composition of meat and milk fat. Feeding ruminants a large amount of unsaturated oils can result in impaired rumen fermentation and inhibition of fibre digestion leading to increased rumen fill (Ikwuegbu and Sutton 1982; Hristov et al. 2005). According to McAllister et al. (1996), long-chain unsaturated fatty acids are directly toxic to protozoa, methanogens and cellulolytic bacteria in the rumen, which can depress fibre digestion and reduce the concentration of ruminal acetic and butyric acids. Moreover, these authors also found

Supported by the Ministry of Science and Higher Education, Poland (Project No. N N311 110138).

doi: 10.17221/9/2017-CJAS

that propionate-producing bacteria were insignificantly inhibited by long-chain fatty acids. They also observed a reduction in the concentration of methane when fatty acids were included in ruminant rations, which mainly resulted from a shift towards propionate production.

Dong et al. (1997) reported a considerable decrease in the abundance of cellulolytic and methanogenic bacteria, which in turn increased the concentration of propionic acid and decreased that of methane, when rumen microorganisms were incubated *in vitro* in the presence of rapeseed oil (which predominantly contains oleic (C18:1) and linoleic (C18:2) acids). Another study showed that the addition of linseed oil (52% C18:3 linolenic acids) to cows' rations decreased the number of cellulolytic bacteria, particularly of *Butyrivibrio fibrisolvens, Ruminococcus albus,* and *Fibrobacter succinogenes* (Yang et al. 2009). Varadyova et al. (2007) demonstrated that the addition of linseed oil to the diet of sheep decreased the count of total protozoa, mainly *Entodinium* spp., but also the family *Isotrichidae*. On the other hand, these authors did not find any difference in the number of protozoa between sheep fed rapeseed oil and control animals.

Ueda et al. (2003) showed a significant interaction between diet composition and linseed oil for ruminal starch degradability. Ruminal disappearance rate of starch decreased along with oil supplementation to the forage-rich ration, whereas it increased when oil was added to the concentrate-rich diet. The addition of linseed oil (which has a high linolenic acid content) to diets of dairy cows significantly decreased the abundance of amylolytic bacteria in the rumen when compared with the addition of soybean oil (which has a high linoleic acid content) to the ration (Yang et al. 2009).

However, the effects of the addition of oil to ruminant diets on bacterial protein synthesis are not clear. Hristov et al. (2005) did not observe increased bacterial protein synthesis when steers were fed a ration containing oleic acid-rich oil, and the supplementation of sheep diets with linseed oil at 26 ml/day resulted in a large increase in the efficiency of bacterial protein synthesis compared to the supplementation at 40 ml/day (Ikwuegbu and Sutton 1982). Yang et al. (2009) showed that the addition of linseed oil to the ration increased the abundance of proteolytic bacteria in the rumen of dairy cows.

We hypothesize that diet supplementation with rapeseed oil, which is rich in oleic acid, or linseed oil, which has a high linolenic acid content, may influence rumen fermentation parameters by modifying the population of different species of protozoa.

The aim of this study was to compare the effect of rapeseed or linseed oils on ruminal disappearance rate of nutrients, ciliate abundance, and the concentration of carbohydrate fermentation products as well as the efficiency of bacterial protein synthesis in the sheep rumen.

MATERIAL AND METHODS

Animals and feed. The experiment was carried out using six female Polish Mountain sheep (mean body weight (BW) = 33 ± 1.1 kg) fitted with cannula to the rumen and a re-entrant cannula to the duodenum, which were placed in the ascending duodenum, anterior to the pancreatic-biliary duct. During the adaptation feeding period (21 days), all animals were housed in individual pens with separate facilities for feeding and watering. Control animals were fed basal diet containing (% of dry matter (DM)) 59.7 meadow hay, 38.2 concentrate, 2.1 vitamin-mineral mixture (Polfamix O-K; Trouw Nutrition Polska, Poland), and the experimental sheep were fed basal ration and 5.2 rapeseed or linseed oils. Compositions of the diets are given in Table 1, and the fatty acid profiles of rapeseed and linseed oils are presented in Table 2. Control and experimental diets were fed in two equal portions at 7:00 and 15:00 h. Oils were stored in the dark at a temperature of 4°C, and the supplements were evenly manually mixed into the concentrate. The average amount of feed provided to animals was 943 g DM/day/sheep. Water was available *ad libitum*. Since the animals were provided with a limited amount of feed, no orts were observed.

All procedures were approved by the Local Ethics Committee for Animal Experimentation.

Experimental design. Six sheep were divided into three groups of two individuals each, which were then assigned to one of three diets (control, rapeseed oil or linseed oil) in a cross-over design with 23-day periods (i.e. 21 days of adaptation and 2 days of sampling). During the sampling period, sheep were maintained in individual metabolic cages.

doi: 10.17221/9/2017-CJAS

Rumen fluid sampling. Samples of rumen fluid were collected from the middle and ventral sacs of the rumen just before feeding and 2, 4, and 8 h after the morning feeding. The samples (approximately 270 ml) were withdrawn by suction using a copper tube ca. 50 cm long and 0.8 cm in external diameter connected to a syringe via a rubber tube. The wall of the sampling end of the copper tube (ca. 5 cm long) had numerous holes ca. 0.2 cm in diameter. The rumen fluid samples were precisely mixed.

Duodenum digesta sampling. Two duodenal cannulas were connected by silicon tubing outside of the body wall. During the sampling period, the cannulas were disconnected, and the duodenal digesta, which passed through the proximal cannula, were quantitatively collected and sampled, and the remaining digesta were returned through the distal cannula. The duodenal digesta were collected continuously for 8 hours, weighed and mixed, and 10% aliquots were pooled and frozen at –20°C until analysis. The digesta were kept at 0°C during collection, and after sampling, they were warmed to 38°C and returned to the intestine through the distal cannula. After the sampling period (8 h), duodenal cannulas were connected by silicon tubing. The duodenal digesta samples were freeze dried before analysis.

Table 1. Composition of diets (% dry matter)

Item	Control diet	Rapeseed or linseed oil diets
Ingredients		
Meadow hay	59.7	56.6
Crushed barley	28.4	26.9
Soyabean oilmeal	9.8	9.3
Oil	–	5.2
Vitamin-mineral premix[1]	2.1	2.0
Chemical composition		
Organic matter	94.1	94.4
Crude protein	15.2	14.4
Crude fibre	20.3	19.2
Crude fat	2.5	7.5
Starch	24.7	23.4
NDF	51.1	48.5
ADF	26.0	24.6
UFV·(per kg)	0.8	0.9

NDF = neutral detergent fibre, ADF = acid detergent fibre, UFV = feed unit of maintenance and meat production

[1]premix contents per kg diet: Vitamin A 300 000 IU, Vitamin D_3 30 000 IU, Vitamin E 1500 mg, Mn 3000 mg, Zn 2500 mg, I 50 mg, Co 15 mg, Se 3 mg, Ca 24%, Na 6%, P 12%, Mg 6.5%

Analytical methods

Protozoa, volatile fatty acids (VFA), and pH. Rumen fluid samples (approximately 20 ml) were passed through four layers of surgical gauze. Samples (5 ml) for counting protozoa were fixed in 4% formaldehyde solution (10 ml) and stored in tightly sealed containers at ca. 4°C. Protozoa were identified and classified according to Dogiel (1927), and counted under a light microscope. Rumen fluid samples for the determination of VFA (5 ml) were preserved with 0.5 ml of formic acid and centrifuged at approximately 11 000 *g* for 25 min. The supernatant was stored in glass vials at ca. 4°C until the determination of VFA concentration, which was analyzed by gas chromatography (GC-2010; Shimadzu, Japan) using a capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) as well as a flame ionization detector (FID) with helium as the carrier gas, according to Miltko et al. (2016). Rumen fluid acidity was measured immediately after collection using a pH-meter (Beckman-Coulter, USA)

Gas production. The concentrations of methane and carbon dioxide were calculated using the molar proportions of volatile fatty acids (acetate, propionate, and butyrate), according to Wolin (1979).

Bacterial mass. The mass of bacteria was measured according to the method described by Michalowski et al. (2003). The suspension (bacterial mass) was weighed, pooled, and stored at –20°C.

DAPA. The 2,6-diaminopimelic acid (DAPA) was used as a specific indicator of bacterial protein synthesis in the rumen, and in this study, the concentration in the duodenal digesta was analyzed using a high-performance liquid chromatography method according to Czauderna and Kowalczyk (1999). The samples of duodenal digesta were

Table 2. Fatty acid contents in rapeseed and linseed oils (g fatty acid methyl esters/100 g oil)

Fatty acid	Rapeseed oil	Linseed oil
C16:0 palmitic	1.9	1.9
C18:0 stearic	2.3	1.6
C18:1n-9 oleic	63.7	28.5
C18:2n-6 linoleic	23.0	17.5
C18:3n-3 linolenic	8.8	50.1

doi: 10.17221/9/2017-CJAS

hydrolysed with 6 M HCl for 24 h at 104°C. The DAPA was determined after derivatization with *o*-phthaldialdehyde in the presence of ethanethiol. Separation of converted DAPA was performed using a column Nova-Pak C-18, 4 µm, 4.6 mm i.d. × 250 mm (Waters Corp., USA) by a binary gradient program and fluorescence detection.

Nutrient analysis. Dry matter (934.01), crude fibre (978.10), crude fat (920.39), starch (920.18), and ash (930.05) in the feedstuff were analyzed according to AOAC (2011) methods. Crude fat and starch in the duodenal digesta were analyzed, too (AOAC 2011), while nitrogen in the feed as well as in duodenal digesta samples was analyzed using the Kjeldahl method (954.01) (AOAC 2011). Natural detergent fibre (NDF) and acid detergent fibre (ADF) in the feedstuff as well as in duodenal digesta were determined according to Van Soest et al. (1991). Organic matter contents were calculated. Fatty acids were converted to fatty acid methyl ester (FAME). The FAME concentration was analyzed by gas chromatography (GC-2010, Shimadzu) using a capillary column (120 m × 0.25 mm i.d. × 0.25 µm film thickness) as well as a flame ionization detector (FID; Shimadzu) with helium as the carrier gas.

Digestibility trial. In the results, disappearance of nutrients until the proximal duodenum is referred to as ruminal disappearance rate.

Statistical analysis. Data were expressed as the mean and pooled standard error of mean values, and the results (protozoa, VFA, pH, and gas production) were subjected to a statistical analysis of variance for a factorial design using the general linear model (GLM). This model included the effect of sheep treatment, sampling time, and the interaction between treatment and sampling time as the main plot as follows:

$$Y_{jkl} = \mu + A_j + Trt_k + St_l + (Trt + St)_{kl} + \varepsilon_{jkl}$$

where:

Y_{jkl} = observation mean
μ = overall mean
A_j = effect of animal (n = 6)
Trt_k = treatment effect (n = 3)
St_l = sampling time effect (n = 4)
$(Trt + St)_{kl}$ = interaction between the treatments and sampling time
ε_{jkl} = random error

The results for the bacterial mass in the rumen, DAPA, nutrients in the duodenal digesta, and digestion in the rumen were determined by variance analysis in accordance with the following linear model:

$$Y_{jk} = \mu + A_j + Trt_k + \varepsilon_{jk}$$

where:

Y_{jk} = observation mean
μ = overall mean
A_j = effect of animal (n = 6)
Trt_k = treatment effect (n = 3)
ε_{jk} = random error

Differences between treatments were assessed by Tukey's HSD post-hoc test using the STATISTICA 10.0 software package. The effects were considered to be significant at $P \leq 0.05$ or $P < 0.01$, and for each effect, two replications under the same experimental conditions were conducted.

RESULTS

Nutrient composition of the diets is presented in Table 1. Daily dry matter intake was higher when oil supplements were fed compared to the control diet (0.97 vs 0.92 kg DM/day); differences in DM intake resulted from the amount of provided feed. As expected, crude fat and feed unit of maintenance and meat production content were greater for oil than control diets. In turn, the content of crude protein and fibre, starch, NDF, ADF (as % of dry matter) was lower for oil than the control rations.

The addition of rapeseed oil to sheep diets caused significantly increased flow of crude protein and ADF to the duodenum when compared to the linseed oil rations (Table 3). The flow of fat to the duodenum was significantly higher in animals that received oils than the control diet.

Supplementation of the ration with rapeseed oil significantly decreased ruminal disappearance rate of crude protein and ADF compared with linseed oil groups (Table 3). Moreover, the addition of rapeseed and linseed oils to sheep diets significantly increased ruminal disappearance rate of fat compared with control animals.

The addition of rapeseed oil to the diets significantly decreased the total number and the concentration of ciliates from the genus *Entodinium* compared with control before feeding and 4 and 8 h after feeding (Table 4). The same oil decreased the concentration of total protozoa and *Entodi-*

doi: 10.17221/9/2017-CJAS

nium spp. 4 and 8 h after feeding compared with animals fed linseed oil. In comparison with those at 0 h, the total count of ciliates and the population density of *Entodinum* spp. were significantly lower at 2 and 4 h after feeding for all groups, and an interaction of significant treatment × sampling time was found for the total count of ciliates and *Entodinium* spp. in the rumen of sheep (Table 4). Population density of *Diplodinium* spp. in the rumen significantly decreased when rapeseed oil was added to the diet compared with control before feeding; the number of species of the genus *Diplodinium* was higher at 0 h compared with 2 and 4 h after the morning feeding in the control group and sheep fed a diet with linseed oil (Table 4). The concentration of protozoa from the genus *Ophryoscolex* was significantly lower in animals fed a ration containing rapeseed oil compared to control before feeding and 8 h after feeding (Table 4). Moreover, compared to the sampling at 0 h, the count of *Ophryoscolex* spp. in the rumen was lower 2 h after feeding for all groups and 4 h after feeding for the control and sheep fed a diet with linseed oil. An interaction of oil supplements and sampling time was detected based on the concentration of *Ophryoscolex* spp. Population density of *Isotricha* spp. decreased 2 and 4 h after feeding in comparison to 0 h for the control and experimental groups, while the population of the genus *Dasytricha* in the rumen was highest in all groups before feeding in comparison to 2 and 4 h after feeding (Table 4). A higher number of *Dasytricha* was observed 2 h after feeding under rapeseed oil supplementation compared to linseed oil.

Supplementation of the ration with oils significantly decreased bacterial mass in the rumen and the concentration of diaminopimelic acid in the duodenal digesta compared with the control group (Table 5).

The pH in the rumen ranged from 5.92 to 6.55 (Table 6), and the lowest pH was observed before feeding in the control group and in the animals fed a diet with rapeseed oil. The highest value was recorded in the linseed oil group at 0 h. There was no univocal tendency towards changes in pH with

Table 3. Intake, duodenal flow, and ruminal disappearance rate of dietary components

Item	Control	Rapeseed oil	Linseed oil	SEM	*P*-value
Intake (g/8 h)					
Dry matter	459.0	484.0	484.0	–	–
Crude protein	69.8	69.8	69.8	–	–
Crude fat	11.3	36.3	36.3	–	–
NDF	234.7	234.7	234.7	–	–
ADF	119.1	119.1	119.1	–	–
Starch	113.3	113.3	113.3	–	–
Flow to duodenum (g/8 h)					
Dry matter	146.5	158.6	170.2	11.46	0.741
Crude protein	41.2^{AB}	41.8^{A}	34.9^{B}	1.13	0.009
Crude fat	9.5^{A}	21.6^{B}	22.4^{B}	1.45	< 0.001
NDF	58.7	54.5	50.2	1.69	0.113
ADF	30.8^{ab}	32.4^{a}	26.7^{b}	0.96	0.038
Starch	18.4	20.1	15.1	0.80	0.059
Ruminal disappearance rate (%)					
Dry matter	68.1	67.2	64.8	2.34	0.866
Crude protein	40.9^{ab}	40.2^{a}	50.0^{b}	1.62	0.040
Crude fat	16.4^{A}	40.5^{B}	38.2^{B}	2.82	< 0.001
NDF	75.0	76.8	78.6	0.72	0.113
ADF	74.1^{ab}	72.8^{a}	77.6^{b}	0.81	0.038
Starch	83.7	82.3	86.7	0.70	0.062

SEM = standard error of the mean, NDF = neutral detergent fibre, ADF = acid detergent fibre
values in rows with different superscripts differ significantly at $^{A,B}P < 0.01$, $^{a,b}P < 0.05$

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Table 4. Concentration of ciliates (× 10^4/ml rumen fluid) in the sheep rumen

Item	Treatment	Hour[1]				SEM	Trt	St	Trt × St[2]
		0	2	4	8				
Total ciliate	control	85.9^{AX}	36.7^{Y}	49.6^{BY}	69.8^{XYa}	1.95	< 0.001	< 0.001	0.002
	rapeseed oil	64.7^{BX}	40.7^{Y}	33.7^{AY}	53.5^{XYb}				
	linseed oil	78.9^{ABX}	30.5^{Y}	45.5^{BY}	66.5^{XYa}				
Entodinium	control	70.4^{AX}	31.2^{Y}	44.0^{BY}	57.4^{AXY}	1.71	< 0.001	0.002	0.003
	rapeseed oil	52.6^{BX}	33.3^{Y}	28.9^{AY}	42.4^{BXY}				
	linseed oil	66.6^{ABX}	25.0^{Y}	40.5^{BY}	56.2^{AXY}				
Diplodinium	control	5.4^{AX}	2.1^{Y}	2.8^{Y}	3.7^{XY}	0.17	0.005	< 0.001	0.063
	rapeseed oil	2.7^{B}	2.4	1.9	3.3				
	linseed oil	4.2^{ABx}	2.1^{y}	2.4^{y}	3.9^{xy}				
Ophryoscolex	control	2.2^{AX}	0.3^{Y}	0.6^{Y}	1.1^{AXY}	0.07	0.003	0.001	0.014
	rapeseed oil	0.8^{Bx}	0.3^{y}	0.5^{xy}	0.4^{Bxy}				
	linseed oil	1.2^{ABX}	0.3^{Y}	0.4^{Y}	0.6^{ABY}				
Isotricha	control	1.1^{X}	0.3^{Y}	0.5^{Y}	2.1^{XY}	0.08	0.520	< 0.001	0.182
	rapeseed oil	1.1^{X}	0.5^{Y}	0.5^{Y}	1.5^{XY}				
	linseed oil	1.2^{X}	0.6^{Y}	0.5^{Y}	1.3^{XY}				
Dasytricha	control	6.8^{Xxy}	2.8^{Yabxy}	1.6^{Yx}	5.5^{XYy}	0.25	0.021	< 0.001	0.543
	rapeseed oil	7.5^{Xxy}	4.2^{Yaxy}	2.0^{Yx}	5.9^{XYy}				
	linseed oil	5.8^{Xxy}	2.6^{Ybxy}	1.9^{Yx}	4.6^{XYy}				

Trt = main effect of treatment, St = main effect of time, SEM = standard error of the mean
[1]samples were taken before morning feeding (at 0 h) and at 2, 4, 8 h after morning feeding
[2]treatment and time interaction effect
different letters in a column ($^{A,B}P < 0.01$) or ($^{a,b}P < 0.05$) show differences between groups (control, rapeseed oil, linseed oil); different letters in a row ($^{X,Y}P < 0.01$) or ($^{x,y}P < 0.05$) show differences between sampling time (0, 2, 4, 8 h)

sampling time, but the values were very similar for all the groups 2, 4, and 8 h after feeding.

As expected, the highest concentration of total VFA was observed 2 and 4 h after feeding and the lowest just before feeding. The concentration of total VFA was lower 2 h after feeding when rapeseed oil was provided compared with control and animals fed linseed oil. In addition, the inclusion of rapeseed oil in the diet for sheep decreased the concentration of acetic acid compared with control 2 h after feeding. Moreover, the concentration of acetic acid was significantly decreased when sheep were fed rapeseed oil compared with those fed linseed oil at 2 and 4 h after feeding. In comparison with the sampling at 0 h, the concentration of acetic acid in the rumen fluid was by ca. 2 mM/100 ml greater at 2 and 4 h after feeding in the control group and sheep receiving linseed oil. Neither of oil supplements influenced the concentration of propionic acid in the rumen (Table 6), for which the highest concentration was also observed at 2 and 4 h after feeding, particularly for the control diet and that with linseed oil. It was found that the concentration of butyric acid in the experimental group was significantly lower than in control before the morning feeding, but linseed oil supplementation significantly increased the level of butyric acid 4 h after feeding relative to the measurement before feeding (Table 6). Linseed oil significantly reduced only the concentration of branched-chain

Table 5. Content of 2,6-diaminopimelic acid (DAPA) in the duodenum digesta (mg/8 h) and bacterial mass in the sheep rumen (g/100 ml/8 h)

Item	Control	Rapeseed oil	Linseed oil	SEM	*P*-value
DAPA	1.45^{a}	0.90^{b}	0.89^{b}	0.09	0.028
Bacteria	1.27^{A}	0.91^{B}	0.90^{B}	0.04	0.001

SEM = standard error of the mean
values in rows with different superscripts differ significantly at $^{A,B}P < 0.01$, $^{a,b}P < 0.05$

doi: 10.17221/9/2017-CJAS

Table 6. Ruminal pH, volatile fatty acids (VFA) concentration and composition (mM/100 ml), and production of fermentation gases (mM/100 ml) in the sheep rumen

Item	Treatment	Hour[1]				SEM	Trt	St	Trt × St[2]
		0	2	4	8				
pH	control	5.97^{X}	6.05^{Y}	6.05^{Y}	6.28^{Y}	0.028	0.257	< 0.001	0.373
	rapeseed oil	5.92^{X}	6.09^{Y}	6.00^{Y}	6.29^{Y}				
	linseed oil	6.55^{X}	6.03^{Y}	6.02^{Y}	6.30^{Y}				
Total VFA	control	9.26^{Yxy}	12.20^{AXabxy}	11.68^{Yx}	9.66^{XYy}	0.162	0.008	< 0.001	0.125
	rapeseed oil	8.85^{y}	10.31^{Bax}	11.10^{x}	9.43^{xy}				
	linseed oil	8.89^{Yxy}	11.45^{ABXbxy}	12.22^{Xx}	9.89^{XYy}				
Acetate	control	5.85^{Y}	7.87^{AXab}	7.63^{Xab}	6.35^{XY}	0.113	0.001	0.002	0.145
	rapeseed oil	5.68	6.48^{Ba}	6.84^{b}	6.10				
	linseed oil	5.81^{Y}	7.55^{ABbX}	8.02^{aX}	6.84^{XY}				
Propionate	control	1.43^{Y}	2.60^{X}	2.32^{X}	1.76^{XY}	0.059	0.904	0.002	0.245
	rapeseed oil	1.48^{y}	2.08^{xy}	2.55^{x}	1.80^{xy}				
	linseed oil	1.60^{Y}	2.30^{X}	2.40^{X}	1.78^{XY}				
Butyrate	control	1.47^{A}	1.37	1.39	1.26	0.027	0.002	0.007	0.154
	rapeseed oil	1.17^{B}	1.34	1.33	1.18				
	linseed oil	1.08^{BY}	1.28^{XY}	1.46^{X}	1.01^{XY}				
Branched chain[3]	control	0.50^{AB}	0.36	0.35	0.29	0.011	0.001	0.058	0.354
	rapeseed oil	0.52^{A}	0.42	0.37	0.35				
	linseed oil	0.40^{B}	0.32	0.35	0.26				
Methane	control	2.45^{y}	3.11^{x}	3.13^{x}	2.87^{xy}	0.052	0.169	< 0.001	0.590
	rapeseed oil	2.45^{Y}	3.10^{X}	3.09^{X}	2.63^{XY}				
	linseed oil	2.32^{Y}	2.98^{X}	3.23^{X}	2.44^{XY}				
Carbon dioxide	control	4.21^{y}	5.33^{x}	5.37^{x}	4.93^{xy}	0.088	0.169	< 0.001	0.590
	rapeseed oil	4.20^{Y}	5.32^{X}	5.30^{X}	4.51^{XY}				
	linseed oil	3.98^{Y}	5.10^{X}	5.54^{X}	4.18^{XY}				

Trt = main effect of treatment, St = main effect of time, SEM = standard error of the mean

[1]samples were taken before morning feeding (at 0 h) and at 2, 4, 8 h after morning feeding

[2]treatment and time interaction effect

[3]isobutyric acid + valeric acid + isovaleric acid

different letters in a column ($^{A,B}P < 0.01$) or ($^{a,b}P < 0.05$) show differences between groups (control, rapeseed oil, linseed oil); different letters in a row ($^{X,Y}P < 0.01$) or ($^{x,y}P < 0.05$) show differences between sampling time (0, 2, 4, 8 h)

fatty acids (isobutyric, valeric, and isovaleric) in the rumen compared with rapeseed oil before feeding.

The estimated methane and carbon dioxide production in the rumen was significantly higher after feeding (2 and 4 h) than before the morning feeding in all groups of sheep (Table 6).

DISCUSSION

Ruminal nutrient digestibility depends on diet composition, source and forms of lipid, as it was suggested by Sutton et al. (1983) and Chelikani et al. (2004). In sheep fed diet with addition of rapeseed oil the ruminal disappearance rate of crude protein was decreased in comparison to the animals receiving linseed oil. Reduction of the apparent ruminal degradability of protein can be explained as a result of higher flow of protein to the duodenum in sheep fed rapeseed oil compared to the animals that received linseed oil. In contrast to our findings, Chelikani et al. (2004) showed an increase in ruminal degradability of crude protein in cows receiving rapeseed oil compared to control animals. In turn, Benchaar et al. (2012) reported no effect of 2%, 3%, and 4% linseed

doi: 10.17221/9/2017-CJAS

oil supplementation to the diet of cows on the total-tract digestibility of crude protein. These authors suggested that the effect of unsaturated fatty acids supplementation, including oils, on protein and other nutrient digestibility varies with the amount of fat added, the source of forage as well as the forage : concentrate ratio of the ration. Furthermore, a lower ruminal disappearance rate of protein may be due to the lower activity of proteolytic bacteria. Szumacher-Strabel et al. (2004) showed that the count of bacteria decreased when ruminal microorganisms were incubated *in vitro* in the presence of rapeseed oil. Moreover, Huws et al. (2015) reported a reduction in the number of proteobacteria in the rumen of steers after supplementation of control diet with echium oil. The bacterial mass in the rumen and DAPA count (bacterial protein synthesis marker) in the duodenal digesta were decreased when sheep received oils in the diet. A higher ruminal microbial degradation of protein in sheep fed linseed oil in the present experiment may be explained by an increased proteolytic activity of the microbes. According to Ikwuegbu and Sutton (1982), the effect of dietary lipids on microbial metabolism probably depends to a considerable degree on the rate of hydrolysis of oil or fat supplements.

The flow of fat to the duodenum and ruminal disappearance rate were higher in sheep fed diets with both oil sources than in those fed the control diet. Elevated flow and ruminal disappearance of fat appears to be the result of higher unprotected (oil) fat intake by the sheep. This is consistent with the results of Oldick and Firkins (2000), who found the microbial degradability of total fatty acids in the rumen of heifers fed vegetable fat (containing C18:2 – 40.1 and C18:1 – 28.5 g/100 g of fatty acids) to be significantly higher compared with the group without oil supplementation. According to Ferlay et al. (1993), the apparent loss of fatty acids within the rumen is caused by absorption from the rumen or metabolism of fatty acids by ruminal epithelium.

Supplementation of diet with rapeseed oil decreased ruminal disappearance rate of ADF compared to sheep fed rations with linseed oil. Lower ADF degradability in the rumen was associated with a higher flow of ADF to the duodenum in sheep fed rapeseed oil compared with animals receiving linseed oil. This was consistent with reduced digestibility of cellulose or crude fibre when free oil was added to various rations (Ikwuegbu and Sutton 1982). The principal effect of rapeseed oil was shifting the site of ADF digestion to the intestines, probably the caecum. The supplementation of the diet with linseed oil increased microbial degradation of ADF in the rumen; this effect is difficult to explain. However, an increase in the ruminal degradation of ADF could result from an increase in total number of protozoa and numerous cellulolytic ciliates from the genus *Diplodinium*, e.g. *Eudiplodinium maggi*, *Diploplastron affine,* and *Anoplodinium denticulatum*, which are commonly present in the rumen of domestic ruminants. It cannot be precluded that the increase in microbial degradation of ADF in the rumen may result from different composition of fatty acids in linseed oil in relation to rapeseed oil. In turn, fatty acids present in linseed oil are probably less toxic for cellulolytic microbes than the components of rapeseed oil. According to Benchaar et al. (2012), the effects of oil supplementation on ruminal disappearance rate depended on the amount of oil added to the diet. The addition of 26 and 40 ml/day of linseed oil to the diet of sheep significantly decreased ruminal disappearance rate of ADF compared with that in animals receiving 13 ml/day oil and a control (0.2 and 0.1 vs 0.3 and 0.4 g/day, respectively) (Ikwuegbu and Sutton 1982). Benchaar et al. (2012) reported a significant decrease in the effective ruminal degradability of timothy hay when dairy cows were fed 4% linseed oil compared with animals that received only 2% oil. Jalc et al. (2006) showed that the addition of fat to the diet depressed rumen fibre degradation through the physical coating of the fibre by oil as well as the inhibition of rumen microbial activity. Ruminal disappearance rate of ADF increased (57.2%) with linseed oil supplementation to the forage-rich diet, whereas it decreased (24.6%) with the supplementation to the concentrate-rich ration for dairy cows (Ueda et al. 2003). These authors found a trend interaction (P = 0.10) between forage : concentrate ratio and linseed oil supplementation for ADF digestibility in the rumen. Sutton et al. (1983) observed a higher ruminal disappearance of fibre fraction when sheep were fed protected linseed oil and protected coconut oil than linseed oil and coconut oil.

The count of total protozoa as well as the number of individual rumen ciliate genera were lower at 2 and 4 h after the morning feeding than before

doi: 10.17221/9/2017-CJAS

this feeding in both control and treatment groups. These results are consistent with findings of Cieslak et al. (2015), who reported that the density of the ciliate population in the rumen of cows significantly decreased 3 and 6 h after feeding a diet containing a mixture of rapeseed and fish oils. Wencelova et al. (2014) showed that the density of total protozoa as well as that of *Entodinium* spp. decreased when hay and barley grains (800 : 200 or 500 : 500 w/w) were incubated with rapeseed oil. Our results are in agreement with those of the authors cited above. The number of species in the genera *Diplodinium* and *Ophryoscolex* decreased before feeding and 8 h after feeding when sheep were fed rapeseed oil; this is consistent with the results of *in vitro* study by Wencelova et al. (2014), who reported that the addition of rapeseed oil decreased the number of *Enoploplastron triloricatum* and *Polyplastron multivesiculatum* (subfamily Diplodininae) as well as *Ophryoscolex c. tricronatus* (subfamily Ophryoscolecinae). Rapeseed oil is rich in unsaturated acids, oleic acid C18:1, and linoleic acid C18:2, and a large amount of unsaturated fatty acids may reduce the ciliate population in the rumen. According to Ivan et al. (2001), prolonged dietary supplementation with a high dose of sunflower seed oil (more than 5% dietary DM, containing C18:1 – 21.1% and C18:2 – 66.2%) was effective at decreasing the number of ciliates in the rumen. These authors observed a lower count of *Entodinum* spp., Isotrichidae (*Isotricha* and *Dasytricha* spp.) as well as cellulolytic protozoa (*Polyplastron, Diplodinium,* and *Metadinium* spp.) when sheep were fed a diet with 6% sunflower seed oil. Moreover, Isotrichidae as well as cellulolytic ciliates disappeared from the rumen of sheep fed an "oil" ration after 10 and 21 days, respectively. Newbold et al. (2015) suggested that ruminal ciliates digest as well as convert dietary fat, but a high lipid concentration in the ration is toxic to protozoa. Szumacher-Strabel (2010) reported that the genus *Epidinium* spp. (*E. ecaudatum* and *E. caudatum*) participated in 30% of lipolysis process, but these ciliates were not identified in our study. According to Newbold et al. (2015) the antiprotozoal effect of fat depends on the fatty acid composition. Medium chain fatty acids are more effective in controlling ciliate amount than polyunsaturated fatty acids. These authors reported that lauric acid (C12:0) may reduce the number of protozoa by 40%. Moreover, Hook et al. (2010) and Newbold et al. (2015) pointed out that other chemicals such as cooper sulfate, alkanates, calcium peroxide, ionophores as well as saponin may be toxic for protozoa. The strong decrease in the number of *Entodinium* spp., when sheep were fed rapeseed oil, was associated with microbial degradation of starch in the rumen ($P = 0.062$). As these microbes can ingest and digest starch, they were probably responsible for the decrease in the degradability of this carbohydrate. Moreover, diet supplementation with rapeseed oil significantly decreased bacterial mass in the rumen. Dong et al. (1997) observed lower densities of total bacteria and amylolytic bacteria in the rumen fluid when rapeseed oil was added compared with a control group (116.0 and 114.0 vs 125.0 and 125.0 × 10^8/ml).

The lower efficiency of bacterial protein synthesis (DAPA was used as a marker in the duodenum digesta) in sheep fed diets containing oil may be explained by the reduction in the population of bacteria in the rumen. Ikwuegbu and Sutton (1982) observed that the addition of 40 g/day linseed oil to sheep diets reduced bacterial protein synthesis, presumably because of an inhibitory effect of linseed oil on bacterial metabolism. In contrast, Oldick and Firkins (2000) suggested that a decrease in the number of total protozoa may increase the efficiency of microbial protein synthesis. We observed a reduction in the total number of protozoa and *Entodinium* spp. as well as lower bacterial protein synthesis when sheep were fed rapeseed oil, but these results are difficult to interpret. It is likely that the high oleic acid (C18:1) content in the rapeseed oil had a negative impact on the population of microorganisms (bacteria and protozoa) in the sheep rumen. The higher number of total ciliates (before feeding and 4 and 8 h after feeding) as well as the low level of bacterial protein synthesis in the rumen of sheep receiving linseed oil could be caused by the consumption of bacteria by protozoa in the rumen or a competitive source of nutrients (Ueda et al. 2003).

The pH of the rumen fluid ranged from 6.50 to 5.92. The pH slightly, but significantly increased after sheep were fed rapeseed oil, while it decreased when animals received linseed oil. This fact is difficult to explain; differences in the level of unsaturation between these two oils probably changed the pH in the rumen. Rapeseed oil contains high amounts of oleic acid – C 18:1n-9 (64.0 g FAME/100 g oil), while linseed oil has more li-

doi: 10.17221/9/2017-CJAS

nolenic acid – C 18:3n-3 (50.0 g FAME/100 g oil). Probably, linolenic acid stabilizes better the acidity in the rumen than oleic acid. Szumacher-Strabel et al. (2009) documented an increase in pH in the rumen fluid of goats fed evening primrose oil (containing 84.6% of polyunsaturated fatty acids and 7.4% of monounsaturated fatty acids) when compared to animals receiving borage oil (containing 48.4% of polyunsaturated fatty acids and 27% of monounsaturated fatty acids). It cannot be ruled out that the increase in rumen pH before feeding linseed oil could have decreased the concentration of lactic acid. Cieslak et al. (2015) did not find significant changes in the pH of the rumen fluid after the addition of a mixture of rapeseed and fish oils to cows' diets, but they observed a significant increase in the acidity of the rumen 3 and 6 h after feeding compared with that observed at 0 h.

The total VFA concentration in the rumen significantly decreased in sheep fed rapeseed oil mainly due to a decline in the concentrations of acetate (2 and 4 h after feeding) and butyrate (before the morning feeding). Our results are consistent with those of Doreau (1992), who observed a greater decrease in the concentration of total VFA as well as acetate and butyrate under rapeseed oil supplementation compared to fish oil in the rumen of dairy cows 2.5 h after feeding. In the current study, the reduction in the concentrations of acetate and butyrate may be due to the depression of the total ciliate population, particularly of *Entodinium* spp., and before the morning feeding, species of the genera *Diplodinium* and *Ophryoscolex.* These protozoa primarily produce acetic and butyric acids (Michalowski 1987), thus it cannot be precluded that the decrease in acetate in the rumen of animals fed the diet supplemented with rapeseed oil was due to a reduction in cellulolytic bacteria population. In the present study, a decrease in the total bacterial biomass was observed. The addition of linseed oil to the diet decreased the concentration of branched-chain fatty acids, which was caused by a lower deamination process in the rumen (Wolin et al. 1997).

A review by Hook et al. (2010) showed that increasing the amount of oil in ruminant diets caused a decrease in methanogenesis as a result of ciliate number reduction, and increased concentration of propionic acid as well as the biohydrogenation of unsaturated fatty acids. In the present study, no influence of oils on the estimated methane production and the concentration of propionate in the sheep rumen was found, but the diet with rapeseed oil reduced protozoan population; these results are consistent with those of Cieslak et al. (2015). McAllister et al. (1996) and Ivan et al. (2001) showed that long-chain fatty acids were directly toxic to protozoa and cellulolytic bacteria population. The oils could indeed have lowered microbial density, but the quantity of oil added was still too low to induce detectable changes in methane formation in the rumen. According to Johnson et al. (2002), the lack of differences in methane production may be the result of insufficient amount of fat in the ration (4–5.6% DM), restricted fat hydrogenation in the rumen as well as decreased ruminal fermentation, or it could be due to interactions between the fat and calcium in ruminant diets. In contrast, Dong et al. (1997) observed decreased methane production with rapeseed oil supplementation, which corresponded with a reduced methanogenic bacterial population and a higher propionic acid concentration in the RUSITEC study. According to this author, unsaturated fatty acids present in the oil may be toxic to methanogenic bacteria, and Dohme et al. (2001) found that fatty acids inhibited methanogen populations by binding to the cell membrane and disrupting membrane transport. Moreover, the addition of oil did not depress ruminal NDF disappearance even though methane emission was reduced (Dong et al. 1997). The diet supplementation with rapeseed oil numerically decreased the estimated concentration of methane (4, 8 h after feeding), but did not affect the microbial degradation of NDF in the rumen compared with control animals. The concentration of methane depends on the type of fat as well as the composition of the ration, particularly the type of carbohydrates, which is consistent with the findings of Dong et al. (1997), who reported a lower methane formation in an artificial rumen system with grass hay and rapeseed oil diet compared to the concentrate (90% wheat plus 10% hay) with the same oil (1.4 vs 2.2 mM/day). These authors observed that the reduction in methane production corresponded with a decrease in the mass of methanogenic bacteria.

CONCLUSION

The rapeseed oil exhibits the greatest potential to suppress ciliate populations and rumen fermenta-

doi: 10.17221/9/2017-CJAS

tion as well as apparent ruminal degradability of ADF and crude protein. Apparently, our results showed that the oleic acid more strongly reduced protozoa and digestive processes in the rumen than linolenic acid. However, it was clear that none of the additives had a dramatic impact on the production of methane. The quantity of oils added was still too low to induce detectable changes in methane formation in the rumen. Future studies are required to better explain the use of oils and fatty acids as a component of ruminant diets and their influence on protozoa turnover in digestive process in the rumen.

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Received: 2017–01–24
Accepted after corrections: 2017–09–21