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In vitro ruminal fermentation and fatty acid production by various oil seeds

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Abstract

Rumen simulating techniques (Rusitec) were used to determine the impact of diets containing milled oilseeds on the fermentation parameters and amount of fatty acids (FA) in the effluent. High-forage diets containing no oilseeds (control diet (CD)) or 10% oilseed meal from rapeseed (RS), sunflower seed (SS), or flaxseed (FS) were used on a dry matter (DM) basis. No differences in DM digestibility were observed between the diets. Inclusion of SS and FS significantly reduced the pH values of the ruminal fluid, and a significant decline in the ammonia nitrogen (NH_3-N) (mg/d) production in effluent was observed in the vessels with SS. Generally, oilseeds in these diets significantly reduced the amount of total fermentation gases (L/d); however, only a tendency toward methane (CH_4, %) decrease was detected. The addition of oilseeds also significantly diminished the amount of total volatile fatty acids (VFA) produced (mmol/d). Significant reductions in the amounts of saturated FA in the vessels with RS and FS were observed compared with the CD and a significantly higher amount of monounsaturated fatty acids (MUFA) was noted in the vessels with RS. An increased amount of polyunsaturated fatty acids (PUFA), compared with the CD, was statistically significant only in the vessels with FS.

Keywords: Flaxseed, high-forage diet, methane, rapeseed, sunflower seed
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Introduction

Fat supplements such as oilseeds are included in the diets of ruminants to increase energy density, improve nutrient utilization, enhance milk and meat yields, and modify fatty acid (FA) composition (Soder *et al*., 2013). Dietary fats can modify the ruminal microbial population responsible for cellulose digestion (Getachew *et al*., 2001), reducing the rumen degradation of fibre and organic matter (Machmüller *et al*., 2006). Nevertheless, some experiments showed no effect (Beauchemin *et al*., 2007) and even positive responses (Sinclair *et al*., 2005) after the addition of oils to the diets. Controversial results may be due not only to the type and amount of oil, but also to the basal diet composition.

The addition of unsaturated fat sources decreased the concentration of ammonia nitrogen (NH_3-N) in the rumen (Egan *et al*., 1986) and enhanced the effectiveness of microbial nitrogen synthesis (Oldick & Firkins, 2000). The addition of fat to the ruminant rations appears to be an efficient and easy way of reducing methane (CH_4) production (Boadi *et al*., 2004). Fats with high amounts of long-chain unsaturated fatty acids, such as sunflower and rapeseed oils, depressed the emissions of CH_4 by approximately 22% of the total energy received by cows fed diets with high contents of forage (McGinn *et al*., 2004; Beauchemin & McGinn, 2006). However, it has been reported that fats do not have any specific effect on CH_4 release, and that the evident decrease reflects a fall in the digestibility of the ration nutrients (Beauchemin *et* al., 2007).

Based on these findings, the authors hypothesized that the addition of milled oilseeds to the high-forage diet is not necessarily associated with detrimental effects on ruminal fermentation. The objectives of the present study were therefore to determine the impact of feeding high-forage diets containing milled oilseeds on the fermentation parameters and amount of FAs in effluents in an *in vitro* experiment.

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Materials and Methods

The incubation was performed using Rumen simulating techniques (Rusitec) with a unit consisting of four vessels with a nominal volume of 850 ml each (Czerkawski & Breckenridge, 1977). The control diet (CD) included 70% cut meadow hay (from 10 to 12 mm long) and 30% barley meal (ground through a 1 mm screen sieve) on a dry matter (DM) basis. Experimental diets included 70% cut meadow hay, 20% barley meal and 10% oilseed meal - rapeseeds (RS), sunflower seeds (SS) or flax seeds (FS) on a DM basis. Seeds were ground through a 1-mm screen sieve. The flow through fermenters was maintained by continuous infusion of McDougall´s solution of artificial saliva supplemented with microelements (mg/L): zinc sulfate (ZnSO), 1.92; magnesium sulfate ($MnSO_4$), 1.02; cobalt sulfate ($CoSO_4$), 0.06; at pH 8.4; (McDougall, 1948) at a rate of 610 mL/d (dilution rate of 3.0%/h; Dohme *et al*., 2000). With the dietary crude protein (CP) contents ranging from 90.0 g/kg for the CD to 111.0 g/kg (on DM basis) for the diets containing oilseeds, 283.2 (CD), 85.0 (RS diet), 160.5 (SS diet), and 122.8 (FS diet) mg of urea per 1 L of McDougall´s solution of artificial saliva was added to balance the CP contents in the respective vessels to 12%.

The duration of the experimental period was 12 days, with the first six days being used for equilibration and the last six days for sample collection. Ruminal fluid used as inoculum was obtained from two ruminally fistulated Slovak merino sheep (mean bodyweight 42.1 $\pm$ 2.0 kg) fed on a diet consisting of 70% meadow hay and 30% barley meal. Sheep were housed in individual pens with free access to water and feed. The experimental protocol was approved by the Ethical Committee of The Institute of Animal Physiology Slovak Academy of Sciences and State Veterinary and Food Office (Ro-2762/05-221/3). Rumen content (solid and liquid) was collected through a ruminal cannula one hour before the morning feeding and transferred to the laboratory under anaerobic conditions at 39 °C. Each reaction vessel was filled with 450 ml rumen fluid and 400 ml artificial saliva. Squeezed particulate rumen contents (100 g) were weighed into a nylon bag (pore size 100 μm), which was placed inside the feed container in each vessel as a donor of bacteria during the first 24 hours. Each vessel contained two bags of the same substrate (introduced on two consecutive days), which remained for 48 hours in the fermenters. The fermenters were purged with nitrogen (N_2) after manipulation to maintain anaerobic conditions, and the temperature in vessels was maintained at 39 °C. During sampling (days 7–12), the effluent collection flasks were cooled to 2 °C in a refrigerated water bath to inhibit microbial growth and fermentation.

Fluid was sampled daily from each vessel before the bags were replaced and the pH (Inolab Level 1, WTW, Weilheim, Germany) was measured immediately. (The pH values on days 7–12 were used for statistical analysis). The gas produced was collected daily on days 7–12 in new rubber bags and analysed for total gas volume and CH_4 concentration. The liquid effluents in the flasks were also collected daily, and on days 7–12 were analysed for volatile fatty acids (VFA), FA and NH_3-N production. The disappearance of DM after 48 hours incubation was determined from the weight loss after oven drying at 55 °C for 48 hours, and the residues were then analysed for neutral detergent fiber (NDF) (Van Soest *et al*., 1991) in the presence of sodium sulphite without α-amylase treatment and the results presented as ash free. The acid detergent fiber (ADF) (AOAC Official Method 973.18, AOAC, 2005), CP (Kjeldahl method), ether extract (EE), and ash (AOAC, 2005) analyses were done on the diets and in the residues to allow for the determination of nutrient digestibility. The VFA concentrations in the effluents were determined by gas chromatography (Cottyn & Boucque, 1968) in a Perkin-Elmer 8500 gas chromatograph using crotonic acid as an internal standard. The NH_3-N concentrations were measured by the microdiffusion method (Conway, 1962), gas volumes were determined with a flow gasometer, and CH_4 was detected in a Perkin-Elmer 8500 gas chromatograph as described by Czerkawski & Clapperton (1984).

The lipids from the diets and effluents were extracted and methylated according to the method of Folch *et al*. (1957). The fatty acid methyl ester profiles were determined by gas chromatography on a 6890N chromatograph (Agilent Technologies) with a G1315A autosampler. A 60 x 0.25-mm x 0.25-μm i.d. fused silica capillary column (DB-23, Agilent Technologies) was used. The N_2 was applied as the carrier gas at 0.8 ml/min and as the makeup gas at 30 ml/min. The temperatures of the injector and the flame ionization detector were 230 °C and 260 °C, respectively. A timed-temperature program was used with the following settings: i) an initial oven temperature of 120 °C with a hold for 6 min; ii) an increase of 15 °C/min to 170 °C; iii) an increase of 3 °C/min to 210 °C, followed by a hold for 13.5 min; and iv) an increase of 40 °C/min to 230 °C, with a final hold for 7 min. The total run time was 44 min. The split ratio was 1:1 for the effluents and 1:40 for the feeds, with 1 μl injected. The total run flow rates of air and hydrogen were 300 ml/min and 30 ml/min, respectively. Data were extracted with a GC ChemStation B.01.01.

The data were analysed statistically using the general linear model (GLM) of SAS Institute, Inc. (2000) for a completely random design with the following model:

$$Y = \mu + T_i + e_{ij},$$

Where: Y is the dependent variable

μ is the overall mean,
T_i is the fixed effect of the dietary treatments (i = 1–4)
e_{ij} is the residual effect

When differences among the vessels were significant at the 0.05 level, Tukey's post hoc test was used to compare the means among treatments. The normality of values was evaluated with the Shapiro-Wilk test (SAS Institute, 2000). The results in the tables are presented as the means and the standard errors of means (SEM).

Results and Discussion

The chemical composition of the components and the diets are shown in Table 1. The oilseeds contained from 182 g/kg to 225 g/kg (on a DM basis) CP compared with 118 g/kg in barley meal. As expected, the fat contents were higher for the oilseed diets than for CD.

Table 1 Ingredients and chemical composition of diets supplemented or not supplemented with oilseeds*

	DM	NDF	ADF	CP	EE
	g/kg	g/kg DM			
Meadow hay	931	624	398	78	12
Barley meal	882	293	76	118	22
Rapeseed (RS)	940	407	349	225	368
Sunflower seed (SS)	954	525	258	182	324
Flax seed (FS)	925	380	257	222	348
Control diet (CD)	909	528	305	90	13
Rapeseed diet (RS diet)	915	505	334	111	48
Sunflower seed diet (SS diet)	931	546	323	103	45
Flax seed diet (FS diet)	927	537	325	107	47

* CD: control diet including 70% meadow hay + 30% barley meal; FS diet: 70% meadow hay + 20% barley meal + 10% flax seeds meal; RS diet: 70% meadow hay + 20% barley meal + 10% rapeseeds meal; SS diet: 70% meadow hay + 20% barley meal + 10% sunflower seeds meal
ADF: acid detergent fibre; CP: crude protein; DM: dry matter; EE: ether extract; NDF: neutral detergent fibre

Table 2 shows the FA profile of the oilseeds and experimental diets. The FS diet contained the highest amount of α-linolenic acid ($C_{18:3}$; 55.8%) among the oilseeds. The content of linoleic acid ($C_{18:2}$) was highest in SS and reached 56.4% of total FA. The RS diet supplied the highest content of oleic acid ($C_{18:1}$; 61% of measured FA). Overall, the concentrations of palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) were low in all oilseeds and ranged from 4.8 to 6.4% for $C_{16:0}$ and from 1.2 to 4.5% for $C_{18:0}$.

No differences (P >0.05) in dry matter digestibility (DMd) were observed between the diets in the present study (Table 3). Similarly, no differences in DMd were observed in diets with RS in experiments by Beauchemin *et al*. (2009) and Zhang *et al*. (2007). However, a decrease in DMd was observed in diets for lactating cows that contained FS or SS (Beauchemin *et al*., 2009) while an increase in DMd was reported in an experiment by Zhang *et al*. (2007), in which lactating ewes were fed a diet containing FS**.** The digestibility of neutral detergent fiber (NDFd; Table 3) was enhanced by the addition of SS and FS to the diet, compared with CD (P <0.001) diets. Inclusion of RS, relative to CD, had no effect on NDFd. The higher digestibility of acid detergent fibre (ADFd) was observed in the SS and FS diets compared with the CD (P <0.001) and RS diets (P <0.001). No differences were found between the CD and RS or between the SS and FS diets. In comparison with CD, feeding FS decreased the digestibility of crude protein (CPd). The low CPd of FS in the rumen of sheep was also reported by Zagorakis *et al*. (2015). According to Gonthier *et al*. (2004), FS supplementation increased post-ruminal digestibility. Additionally, FS had high fibre fraction digestibility (Gonthier *et al*., 2004; Zagorakis *et a*l., 2015). The addition of oilseeds to the diet enhanced the digestibility of fat (EEd) compared with CD (P <0.001). Because of the higher amount of EE in oilseeds, the higher EEd in experimental diets was expected. In the present study, the authors did not confirm the negative effect of the addition of oilseeds to the high forage diets on the digestibility of nutrients. According to Moran (2005),

dietary lipid content of about 5% should not cause harmful imbalance to the rumen environment and is not likely to affect digestibility negatively.

Table 2 Fatty acid profile (%) of feeds and diets supplemented or not supplemented with oilseeds*

	Ingredients of diets					Experimental diets			
	Meadow hay	**Barley**	**RS**	**SS**	**FS**	**CD**	**RS diet**	**SS diet**	**FS diet**
$C_{16:0}$	19.7	19.6	4.8	6.4	6.2	20.4	10.6	9.8	9.0
$C_{18:0}$	5.6	1.8	1.2	4.5	3.7	2.2	1.8	4.1	4.8
$C_{18:1\text{-}n9}$	38.2	20.6	61.0	31.0	15.8	7.4	39.4	24.4	17.2
$C_{18:2\text{-}n6}$	14.8	48.3	18.7	56.4	17.1	31.1	22.2	49.7	16.7
$C_{18:3\text{-}n3}$	12.4	6.5	8.7	0.1	55.8	32.9	19.6	9.3	50.2
Others	9.3	3.2	5.6	1.6	1.4	6.0	6.4	2.7	2.1
SFA	28.3	22.2	6.7	11.3	10.2	24.1	13.6	14.6	14.7
MUFA	42.1	23.0	65.8	31.4	16.7	10.0	43.6	25.5	18.3
PUFA	29.6	54.8	27.5	57.3	73.1	65.9	42.8	59.9	67.0

* CD: control diet including 70% meadow hay + 30% barley meal; FS diet: 70% meadow hay + 20% barley meal + 10% flax seeds meal; RS diet: 70% meadow hay + 20% barley meal + 10% rapeseeds meal; SS diet: 70% meadow hay + 20% barley meal + 10% sunflower seeds meal
MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SEM: standard error of mean; SFA: saturated fatty acids
$C_{16:0}$: palmitic acid; $C_{18:0}$: stearic acid; $C_{18:1\text{-}n9}$: oleic acid; $C_{18:2\text{-}n6}$: linoleic acid; $C_{18:3\text{-}n3}$: linolenic acid

Table 3 Effects of diets supplemented or not supplemented with oilseeds* on the nutrients digestibility (g/kg) in 48 hours in *in vitro* experiment, averages of days 7–12

Variable	**Experimental diets**				**SEM**	***P*-value**
	CD	**RS diet**	**SS diet**	**FS diet**		
DMd	557	519	571	533	44.1	NS
NDFd	325[a]	324[a]	520[c]	411[b]	49.4	<0.001
ADFd	278[a]	257[a]	477[b]	448[b]	60.5	<0.001
CPd	613[b]	660[b]	641[b]	493[a]	40.3	<0.001
EEd	396[a]	476[a]	699[b]	509[a]	64.3	<0.001

* CD: control diet including 70% meadow hay + 30% barley meal; FS diet: 70% meadow hay + 20% barley meal + 10% flax seeds meal; RS diet: 70% meadow hay + 20% barley meal + 10% rapeseeds meal; SS diet: 70% meadow hay + 20% barley meal + 10% sunflower seeds meal
ADFd: acid detergent fibre digestibility; CPd: crude protein digestibility; DMd: dry matter digestibility; EEd: ether extract digestibility; NDFd: neutral detergent fibre digestibility; SEM: standard error of mean
[a, b, c] Values within a row with different superscripts differ (P <0.05)

The addition of SS to the diet lowered the production of NH_3-N (mg/d) in the effluent, compared with CD and RS diets (P <0.001; Table 4). The production of volatile fatty acids (VFA) (mmol/d) was lowered by the addition of oilseeds to the diet (P <0.001) (Table 4). The lowest production of VFA was observed in vessels with FS diet in comparison with other diets (P < 0.001). The FS added to the diet also decreased the production of acetate and butyrate, compared with other oilseed diets and CD (P <0.001). Additionally, the lowest acetate : propionate ratio was observed in vessels with RS and FS diets, compared with CD and SS diets (P <0.001). The FS affected the rumen fermentation pattern in a manner similar to that shown by Sutton *et al*. (1983) in a study with sheep, with a lower VFA concentration and smaller acetate : propionate ratio. This would indicate disturbed ruminal fibre degradation in the rumen (Jenkins, 1987), but the higher digestibility of fibre fractions was observed in our study. Simultaneously, the production of methane was not

decreased (see below), thus the authors assume no reduction of microbial population and utilization of fiber fermentation products for creation of microbial biomass (Demeyer, 1991). Acetate : propionate ratio of 3.0-4.1 was reported in vivo and 2.0-4.1 in vitro in an experiment by Brown *et al*. (2002). High acetate : propionate ratio is an indication of proportionally higher digestible NDF in the feeds.

Generally, the addition of oilseeds to the high-forage diet reduced the amount of total fermentation gases (L/d) compared with CD, particularly for SS (P <0.05) and FS (P <0.01; Table 4). A positive correlation between gas and VFA production was detected by Getachew *et al*. (2004). Fermentative gas is produced mainly when feedstuffs are fermented to acetate and butyrate. However, although a decline in the production of fermentation gases was observed, the authors detected only a tendency for CH_4 (%) decrease (P =0.06). Martin *et al*. (2008) fed various forms of flaxseeds (5.7% added fat) to dairy cows and reported a 10% reduction in CH_4 (g/kg of dry matter intake (DMI)) for unprocessed seeds and a 49% reduction for the crude oil of the seeds. McGinn *et al*. (2004) added 5% sunflower oil to a forage-based diet for beef cattle and reduced CH_4 (g/kg of DMI) by 17%. Beauchemin *et al*. (2007) added sunflower oil and whole sunflower seeds (3.4% added fat) to a forage-based diet fed to beef cattle and observed a 15% reduction in CH_4 production. However, no changes in CH_4 production were observed by Johnson *et al*. (2002), who added up to 5.6 % fat from a mixture of RS and whole cottonseeds to the diet for dairy cows. Similar results were obtained by Woodward *et al*. (2006), who fed dairy cows on pasture with a mixture of FS and fish oils. The literature indicates that supplemental fats could reduce CH_4 emissions, but in many cases CH_4-suppressing effects were influenced by the types of forage (maize silage versus hay) (Chung *et al*., 2011), and the amount of added fat (% in diet) and its treatment (Martin *et al*., 2008).

Table 4 Effects of diets supplemented or not supplemented with oilseeds* on the daily production of volatile fatty acids, ammonia nitrogen and gas production in *in vitro* experiment, averages of days 7–12

Variable	Experimental diets				SEM	*P*-value
	CD	RS diet	SS diet	FS diet		
NH_3-N (mg/d)	120.5^{b}	120.9^{b}	68.2^{a}	90.8^{ab}	19.2	<0.001
VFA production (mmol/d)						
Total	34.5^{d}	30.1^{c}	26.3^{b}	21.4^{a}	2.1	<0.001
Acetate	19.7^{c}	16.1^{b}	16.2^{b}	13.1^{a}	1.4	<0.001
Propionate	6.6^{c}	6.2^{bc}	5.5^{ab}	5.0^{a}	0.5	<0.001
Butyrate	5.9^{d}	5.2^{c}	3.4^{b}	2.6^{a}	0.3	<0.001
Isobutyrate	0.03^{b}	0^{a}	0.13^{c}	0.01^{ab}	0.02	<0.001
Valerate	1.3^{c}	1.5^{d}	0.4^{b}	0.3^{a}	0.08	<0.001
Isovalerate	0.48^{a}	0.89^{b}	0.55^{a}	0.42^{a}	0.09	<0.001
Capronate	0.50^{c}	0.26^{b}	0.13^{a}	0.13^{a}	0.06	<0.001
Acetate : propionate	3.00^{b}	2.60^{a}	2.96^{b}	2.64^{a}	0.23	<0.001
Gas production						
Total (L/d)	3.58^{b}	3.22^{ab}	3.16^{a}	3.07^{a}	0.6	<0.05
CH_4 (ml/d)	119.7	80.5	116.2	106.9	6.1	NS
CH_4 concentration (%)	3.33	2.52	3.67	3.47	0.81	NS

* CD: control diet including 70% meadow hay + 30% barley meal; FS diet: 70% meadow hay + 20% barley meal + 10% flax seeds meal; RS diet: 70% meadow hay + 20% barley meal + 10% rapeseeds meal; SS diet: 70% meadow hay + 20% barley meal + 10% sunflower seeds meal

CH_4: methane; NH_3-N: ammonia-nitrogen; SEM: standard error of mean; VFA: volatile fatty acids

$^{a, b, c}$ Values within a row with different superscripts differ (P <0.05)

The inclusion of oilseeds reduced the pH values of the ruminal fluid in the vessels of SS and FS diets compared with those in the CD (P <0.01 and P <0.001, respectively) and RS diets (P <0.001; Table 5). No differences in pH were observed between the CD and RS diets or between the SS and FS diets. Despite these variations, the mean pH values remained within the physiological range (Krause & Oetzel, 2006). Martínez *et al*. (2010) compared pH values in rumen of sheep and Rusitec for a high-forage diet. The

authors observed that ruminal pH before feeding and mean values of pH over the 12 hours post feeding were similar in both systems (6.69 and 6.45 in sheep and 6.57 and 6.32 in Rusitec). Following these results, the authors can conclude that oilseeds added to the high-forage diets should not have detrimental effects on pH values in the rumen of sheep. A decline in the NH_3-N concentration (mg/L) in the vessel with SS, particularly in comparison with the CD and RS diets (P <0.001) (Table 5) was observed in the current study. According to Shingfield *et al*. (2008), adding SS oil to the diets of lactating cows tends to reduce the NH_3-N concentration in the rumen. The NH_3-N content in the liquid effluent from the vessels varied among diets from 45 to 207 mg/L in Boguhn *et al*.'s (2006) experiment, but the ammonia concentrations were greater than the 100 mg/L reported by Van Soest (1994) as the optimal concentration for efficient amino acid synthesis and microbial growth. Brito *et al*. (2006) suggested that a ruminal NH_3-N concentration below 85 mg/L could depress microbial N synthesis in the rumen. In the present study, the mean NH_3-N concentration ranged from 117 to 193 mg/L and indicates that NH_3-N availability did not impair microbial growth. The addition of oilseeds diminished the amount of total VFA concentration (mmol/L) (Table 5). The lowest VFA concentration was detected in vessels with FS (P <0.001), while SS and RS also lowered the concentration of VFA, compared with CD (P <0.001 and P <0.01, respectively). Leupp *et al*. (2006) noted no changes in total VFA concentration on the addition of rapeseeds to the diets of steers fed with switchgrass hay. Compared with the CD and RS diets, the use of SS and FS enhanced the molar proportion of acetate and reduced the molar proportion of butyrate. All oilseeds, however, enhanced the molar proportion of propionate in effluent, compared with CD. Compared with other studies, the molar proportions of individual VFA, determined in Rusitec in the current study, were within the range determined by Martínez *et al*. (2010) for ruminants.

Table 5 Effects of diets supplemented or not supplemented with oilseeds* on *in vitro* fermentation traits, averages of days 7–12

Variable	Experimental diets				SEM	*P*-value
	CD	RS diet	SS diet	FS diet		
pH	6.67^{b}	6.74^{b}	6.52^{a}	6.55^{a}	0.02	<0.001
NH_3-N (mg/L)	193.1^{b}	192.6^{b}	117.5^{a}	146.2^{ab}	8.8	<0.001
VFA concentration						
Total (mmol/L)	55.3^{c}	48.1^{b}	45.6^{b}	34.5^{a}	1.7	<0.001
Acetate (mol/100 mol)	56.9^{a}	53.4^{a}	61.6^{b}	61.3^{b}	0.8	<0.001
Propionate (mol/100 mol)	19.1^{a}	20.6^{b}	20.9^{b}	23.3^{c}	1.0	<0.001
Butyrate (mol/100 mol)	17.2^{b}	17.3^{b}	12.8^{a}	12.2^{a}	1.4	<0.001
Isobutyrate (mol/100 mol)	0.09^{b}	0.00^{a}	0.48^{c}	0.02^{ab}	0.02	<0.001
Valerate (mol/100 mol)	3.8^{b}	4.9^{c}	1.7^{a}	1.4^{a}	0.4	<0.001
Isovalerate (mol/100 mol)	1.4^{a}	3.0^{c}	2.1^{b}	1.9^{b}	0.5	<0.001
Capronate (mol/100 mol)	1.5^{c}	0.9^{b}	0.5^{a}	0.6^{ab}	0.2	<0.001

* CD: control diet including 70% meadow hay + 30% barley meal; FS diet: 70% meadow hay + 20% barley meal + 10% flax seeds meal; RS diet: 70% meadow hay + 20% barley meal + 10% rapeseeds meal; SS diet: 70% meadow hay + 20% barley meal + 10% sunflower seeds meal

NH_3-N: ammonia-nitrogen; SEM: standard error of mean; VFA: volatile fatty acids

$^{a, b, c}$ Values within a row with different superscripts differ (P <0.05)

The addition of oilseeds to the diets affected the compositions of the fatty acids in the effluents (Table 6). The authors observed a decrease in the amount of saturated fatty acids (SFA) in the vessels with RS (P <0.05) and FS (P <0.001) compared with CD, but a higher amount of monounsaturated fatty acids (MUFA) was noted in the vessel with RS compared with the CD (P <0.001), SS (P <0.01), and FS diets (P <0.01). An increased amount of polyunsaturated fatty acids (PUFA) was detected only in the vessel with FS compared with CD (P <0.01) and RS (P <0.05). The higher ratio values (w3/w6) were observed in vessels with RS. The quantity of lauric acid ($C_{12:0}$) was reduced only in FS compared with CD (P <0.05), and myristic acid ($C_{14:0}$) was decreased by the addition of SS and FS compared with CD (P <0.05). The amount of stearic acid ($C_{18:0}$), the end product of the biohydrogenation of unsaturated FA, was not influenced by the addition of

oilseeds to the diets. The highest amount of oleic acid ($C_{18:1}$) was found in the vessel with RS (P <0.001), which reflects the higher amount of this FA in this oilseed. The vessel with FS contained a higher amount of linoleic acid ($C_{18:2,\ n-6}$) compared with the CD (P <0.01) and RS diets (P <0.05) and a higher amount of linolenic acid ($C_{18:3,\ n-3}$) compared with the CD (P <0.05) and SS diets (P <0.01). Loor *et al*. (2002) have shown that $C_{18:1}$, $C_{18:2,\ n-6}$ and $C_{18:3,\ n-3}$ from dietary rapeseed and soybean oils are converted primarily to $C_{18:0}$ and trans-$C_{18:1}$ isomers in ruminal fluid as a result of PUFA biohydrogenation by rumen microbes. The amounts of *trans*-vaccenic acid ($C_{18:1,\ n-11t}$) and c-9, t-11 CLA were not influenced by the addition of oilseeds; only the amount of t-10, c-12 CLA was decreased by the addition of SS and FS ($P < 0.001$) compared with the CD and RS diet. The amounts of $C_{18:1,\ n-11t}$ and c-9, t-11 CLA were not influenced in the ruminal fluids of fistulated cows fed FS in experiments by Côrtes *et al*. (2010) and the amount of c-9, t-11 CLA in the chymus was not influenced by the addition of sunflower oil to the diets of steers (Sackmann *et al*., 2003). Beaulieu *et al*. (2002) and Duckett *et al*. (2002) detected a higher amount of t-10, c-12 CLA after the addition of oil to diets with high portions of concentrates.

Table 6 Effects of diets supplemented or not supplemented with oilseeds* on amount (%) of selected fatty acids in effluent, averages of days 7- 12

	Dietary treatment				SEM	*P*-value
	CD	RS diet	SS diet	FS diet		
$C_{12:0}$	2.1^{b}	1.1^{ab}	1.0^{ab}	0.8^{a}	0.2	<0.05
$C_{14:0}$	6.8^{b}	3.9^{ab}	3.8^{ab}	3.4^{a}	0.4	<0.05
$C_{16:0}$	35.3^{b}	26.2^{a}	24.4^{a}	21.5^{a}	1.4	<0.001
$C_{18:0}$	15.0	13.9	11.2	9.3	0.8	NS
$C_{18:1\text{-}n11t}$	1.6	2.6	2.7	2.4	0.2	NS
$C_{18:1\text{-}n9}$	17.4^{a}	28.3^{b}	15.6^{a}	18.6^{a}	1.1	<0.001
$C_{18:2\text{-}n6}$	6.3^{a}	8.2^{a}	17.9^{ab}	27.4^{b}	2.5	<0.01
$C_{18:3\text{-}n3}$	0.81^{ab}	2.23^{bc}	0.59^{a}	2.59^{c}	0.28	<0.01
$C_{18:2\ (9,11)}$	0.09	0.21	0.58	0.56	0.07	NS
$C_{18:2\ (10,12)}$	1.23^{b}	1.05^{b}	0.26^{a}	0.11^{a}	0.14	<0.001
SFA	66.7^{b}	50.9^{a}	56.1^{ab}	42.4^{a}	2.5	<0.01
MUFA	22.6^{a}	35.7^{b}	22.1^{a}	25.0^{a}	1.3	<0.001
PUFA	10.7^{a}	13.5^{a}	21.8^{ab}	32.5^{b}	2.6	<0.01
w3/w6	0.09^{a}	0.21^{b}	0.09^{a}	0.12^{a}	0.07	<0.05

* CD: control diet including 70% meadow hay + 30% barley meal; FS diet: 70% meadow hay + 20% barley meal + 10% flax seeds meal; RS diet: 70% meadow hay + 20% barley meal + 10% rapeseeds meal; SS diet: 70% meadow hay + 20% barley meal + 10% sunflower seeds meal

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids

$C_{12:0}$: lauric acid; $C_{14:0}$: myristic acid; $C_{16:0}$: palmitic acid; $C_{18:0}$: stearic acid; $C_{18:1\text{-}n11t}$: *trans*-vaccenic acid; $C_{18:1\text{-}n9}$: oleic acid; $C_{18:2\text{-}n6}$: linoleic acid; $C_{18:3\text{-}n3}$: linolenic acid; $C_{18:2\ (9,11)}$ and $C_{18:2\ (10,12)}$: isomers of CLA (conjugated linoleic acid)

[a, b, c] Values within a row with different superscripts differ (P <0.05)

Conclusions

In the present study, up to 4.8% total fat did not affect the digestibility of nutrients negatively; the mean pH values remained within the physiological range. Unfortunately, only a tendency to CH_4 decrease was detected. The values of FAs in effluent in vessels were influenced by the composition of diets. The addition of oilseeds, mainly RS and FS, decreased the amount of saturated FA and enhanced (only numerically, except vessels with FS) the amount of polyunsaturated FA in effluent. The SS and FS in diets enhanced the digestibility of fibre fractions; despite of this, the production of VFAs decreased. These data, which were obtained from an *in vitro* experiment, indicated that adding oilseeds to the high-forage diet did not have negative effects on the rumen microbial population.

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Authors' Contributions

PK and PH designed the study while its implementation, sample collection and data analysis were done by PK and DJ. PK and FJ participated in results, statistics and interpretation. PK wrote the draft manuscript, while DJ, FJ and PH edited it.

Conflict of Interest Declaration

The authors declare that they have no conflicts of interests with regard to this work.

References

AOAC (Association of Analytical Chemists), 2005. Official methods of analysis. 18th edition. AOAC, Gaithersburg, USA.

Beauchemin, K.A. & McGinn, S.M., 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. J. Anim. Sci. 84, 1489-1496.

Beauchemin, K.A., McGinn, S.M. & Petit, H.V., 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. Can. J. Anim. Sci. 87, 431-440.

Beauchemin, K.A., McGinn, S.M., Benchaar, C. & Holtshausen, L., 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci. 92, 2118-2127.

Beaulieu, A.D., Drackley, J.K. & Merchen, N.R., 2002. Concentrations of conjugated linoleic acid (cis-9 trans-11 octadecadienoic acid) are not increased in tissue lipids of cattle fed a high concentrate diet supplemented with soybean oil. J. Anim. Sci. 80, 847-861.

Boadi, D., Benchaar, C., Chiquette, J. & Massé, D., 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84, 319-335.

Boguhn, J., Kluth, H. & Rodehutscord, M., 2006. Effect of total mixed ration composition and efficiency of ruminal microbial crude protein synthesis *in vitro*. J. Dairy Sci. 89, 1580-1591.

Brito, A.F., Broderick, G.A. & Reynal, S.M., 2006. Effects of varying dietary ratios of alfalfa silage to corn silage on omasal flow and microbial protein synthesis in dairy cows. J. Dairy Sci. 89, 3939-3953.

Brown, V.E., Rymer, C., Agnew, R.E. & Givens, D.I., 2002. Relationship between in vitro gas production profiles of forages and in vivo rumen fermentation patterns in beef steers fed those forages. Anim. Feed Sci. Technol. 98, 13-24.

Chung Y.H., He, M.L., McGinn, S.M., McAllister, T.A. & Beauchemin, K.A., 2011. Linseed suppresses enteric methane emissions from cattle fed barely silage, but not from those fed grass hay. Anim. Feed Sci. Technol. 166-167, 321-329.

Conway E.J., 1962. Microdiffusion analysis and volumetric error. 5th edition. Crosby Lockwood, London, UK. 322 pp.

Côrtes, C., da Silva-Kazama, D.C., Kazama, R., Gagnon, N., Benchaar, C., Santos, G.T.D. & Zeoula, L.M., 2010. Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. J. Dairy Sci. 93, 3146-3157.

Cottyn, B.G. & Boucque, C.V., 1968. Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. J. Agric. Food Chem. 16, 105-107.

Czerkawski, J.W. & Breckenridge, G., 1977. Design and development of a long-term rumen simulation technique (Rusitec). Br. J. Nutr. 38, 371-384.

Czerkawski, J.W. & Clapperton, J.L., 1984. Fats as energy-yielding compounds in the ruminant diet. In: J. Wiseman (ed). Fats in animal nutrition. Butterworths, Boston, MA. pp. 249.

Demeyer, D.I., 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. In: Jouany, J.P., Ed., Rumen microbial metabolism and ruminant digestion. INRA, Paris, pp. 217-237.

Dohme S., Machmüller, A., Wasserfallen, A. & Kreuzer, W., 2000. Comparative efficiency of various fats rich in medium chain fatty acids to suppress ruminal methanogens as measured with Rusitec. Can. J. Anim. Sci. 80, 473-482.

Duckett, S.K., Andrae, J.G. & Owens, F.N., 2002. Effect of high oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. 80, 3353-3360.

Egan, A.R., Boda, K. & Varady, J., 1986. Regulation of nitrogen metabolism and recycling. In: L.P. Milligan, W.L. Grovum, W.L. & A. Dobson (eds). Control of digestion and metabolism in ruminants. Prentice Hall, Englewood Cliffs, NJ. pp. 386-402.

Folch, J., Lees, M. & Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biolog. Chem. 226, 497-509.

Getachew, G., DePeters, E.J., Robinson, P.H. & Taylor, S.J., 2001. In vitro rumen fermentation and gas production: influence of yellow grease, tallow, corn oil and their potassium soaps. Anim. Feed Sci. Technol. 93, 1-15.

Getachew, G., Robinson, P.H., DePeters, E.J. & Taylor, S.J., 2004. Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Anim. Feed Sci. Technol. 111, 57-71.

Gonthier, C., Mustafa, A.F., Berthiaume, R., Petit, H.V., Martineau, R. & Ouellet, D.R., 2004. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. J. Dairy Sci, 87, 1854-1863.

Johnson, K.A., Kincaid, R.L., Westberg, H.H., Gaskins, C.T., Lamb, B.K. & Cronrath, J.D., 2002. The effect of oilseeds in diets of lactating cows on milk production and methane emissions. J. Dairy Sci. 85, 1509-1515.

Krause, K.M. & Oetzel, G.R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Technol. 126, 215-236.

Leupp, J.L., Lardy, G.P., Soto-Navarro, S.A., Bauer, M.L. & Caton, J.S., 2006. Effects of canola seed supplementation on intake, digestion, duodenal protein supply, and microbial efficiency in steers fed forage-based diets. J. Anim. Sci. 84, 499-507.

Loor, J.J., Bandara, A.B.P.A. & Herbein, J.H., 2002. Characterisation of 18:1 and 18:2 isomers produced during microbial biohydrogenation of unsaturated fatty acids from canola and soybean oil in the rumen of lactating cows. J. Anim. Physiol. Anim. Nutr. 86, 422-434.

Machmüller, A., 2006. Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. Agric. Ecosys. Environ. 112, 107-114.

Martin, C., Rouel, J., Jouany, J.P., Doreau, M. & Chilliard, Y., 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86, 2642-2650.

Martínez, M.E., Ranilla, M.J., Tejido, M.L., Ramos, S. & Carro, M.D., 2010. Comparison of fermentation of diets of variable composition and microbial populations in the rumen of sheep and Rusitec fermenters. I. Digestibility, fermentation parameters, and microbial growth. J. Dairy Sci. 93, 3684-3698.

McDougall, E.I., 1948. Studies of rumen saliva. 1. The composition and output of sheep´s saliva. Biochem. J. 43, 99-109.

McGinn, S.M., Beauchemin, K.A., Coates, T. & Colombatto, D., 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and furmaric acid. J. Anim. Sci. 82, 3346-3356.

Moran, J., 2005. How the rumen works. In: Moran, J., Ed., Tropical dairy farming. Landlinks Press, Collingwood, VIC, Australia. p. 41-49.

Oldick, B.S. & Firkins, J.L., 2000. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. J. Anim. Sci. 78, 2412-2420.

Sackmann, J.R., Duckett, S.K., Gillis, M.H., Realini, C.E., Parks, A.H. & Eggelston, R.B., 2003. Effects of forage and sunflower oil levels on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finished diets. J. Anim. Sci. 81, 3174-3181.

SAS (Statistical Analysis Systems), 2000. User´s guide (version 8.1). SAS Institute Inc., Raleigh, North Carolina, USA.

Shingfield, K.J., Ahvenjärvi, S., Toivonen, V., Vanhatalo, A., Huhtanen, P. & Griinari, J.M., 2008. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. Br. J. Nutr. 99, 971-983.

Sinclair, L.A., Cooper, S.L., Chikunya, S., Wilkinson, R.G., Hallett, K.G., Enser, M. & Wood, J.D., 2005. Biohydrogenation of n-3 polyunsaturated fatty acids in the rumen and their effects on microbial metabolism and plasma fatty acid concentrations in sheep. Anim. Sci. 8, 239-248.

Soder, K.J., Brito, A.F. & Rubano, M.D., 2013. Short communication: Effect of oilseed supplementation of an herbage diet on ruminal fermentation in continuous culture. J. Dairy, Sci. 96, 2551-2556.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2nd edition. Cornell University Press, Ithaca, NY, USA.

Woodward, S L., Waghorn, G.C. & Thomson, N.A., 2006. Supplementing dairy cows with oils to improve performance and reduce methane – Does it work? Proc. N. Z. Soc. Anim. Prod. 66, 176-181.

Zagorakis, K., Liamadis, D., Milis, Ch., Dotas, V. & Dotas, D., 2015. Nutrient digestibility and in situ degradability of alternatives to soybean meal protein sources for sheep. Small Rumin. Res. 67, 307-311.

Zhang, R.H., Mustafa, A.F. & Zhao, X., 2007. Effects of feeding oilseeds on nutrient utilization by lactating ewes. Small Rumin. Res. 67, 307-311.