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Effect of diets with fruit oils supplements on rumen fermentation parameters, fatty acid composition and methane production *in vitro*

A. Cieślak[1,3], Z. Váradyová[2], S. Kišidayová[2], D. Jalč[2], and M. Szumacher-Strabel[1]

[1] Poznań University of Life Sciences, Department of Animal Nutrition and Feed Management
Wołyńska 33, 60-637 Poznań, Poland
[2] Institute of Animal Physiology, Slovak Academy of Sciences Šoltésovej 4-6, 040 01 Košice, Slovakia

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ABSTRACT. Effects of diets supplemented with fruit seed oils on fermentation parameters, ciliated protozoan population, and fatty acid composition of the rumen fluid of dairy cows were studied in 24 h batch cultures. Two diets, one containing lucerne plus wheat meal (60:40%) and the other containing of meadow hay plus wheat meal (60:40%), were supplemented with either grape oil or black currant oil (50 g · kg^{-1} of dry matter). The control diet contained no oil supplementation. The oils were selected due to high content of linoleic acid (grape oil, 696 g · kg^{-1} of fatty acids; black currant oil, 586 g · kg^{-1} of fatty acids). Oil supplements did not affect the basal parameters of rumen fermentation. Interactions between the diets and oil supplements affected rumen methane production and either the total or the majority of rumen ciliate species examined. Although the diets had no effect on the total content of volatile fatty acids, the proportions of *n*-butyrate and *iso*-valerate were significantly affected. The concentration of polyunsaturated fatty acids was higher in the meadow hay diet than in the lucerne diet, whereas addition of oils increased polyunsaturated fatty acids content. Black currant oil supplementation proved to be more efficient in enhancing polyunsaturated fatty acids content in rumen fluid when compared to grape oil. In conclusion, both oil supplements considerably decreased methane production when lucerne was used as the diet, what could be the effect of detrimental influence of the type of diet and oil supplement on protozoa population. However, the supplements did not negatively affect other rumen parameters, hence may be considered as valuable supplements in ruminant nutrition.

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Corresponding author:
e-mail: adamck@jay.up.poznan.pl

Introduction

Seeds of berry plants (e.g. *Oenothera biennis*, *Borago officinalis; Cannabis sativa*) and their oils(evening primrose, borage, and hemp oil respectively) could be used as one of the possible sources PUFA (polyunsaturated fatty acids) omega-3 (α-linolenic acid, C18:3*n*-3; eicosapentaenic acid, C20:5; docosahexaenoic acid, C22:6) and omega-6 (linoleic acid, C18:2; γ-linolenic acid, C18:3*n*-6; arachidonic acid, C20:4) fatty acids in ruminant nutrition. Oil from berry seeds is gaining increasing attention also due to high content of essential fatty acids (FA) and antioxidants (Van Hoed et al., 2009). It is known that antioxidants of grape and black currant seeds are valuable sources of bioactive phenolics and their composition is interesting from a nutritional point

of view (Lu and Foo, 2003; Shi et al., 2003). Grape (*Vitis vinifera*) oil (GO) contains linoleic acid (600–750 g · kg^{-1} of FA), oleic acid (140–220 g · kg^{-1} of FA) and α-linolenic acid (10 g · kg^{-1} of FA; Dubois et al., 2007; Matthäus, 2008). Black currant (*Ribes nigrum*) oil (BCO) has a similar content of either linoleic, oleic or γ-linolenic acids (160–170 g · kg^{-1} of FA) and comprises more α-linolenic acid (120 –130 g · kg^{-1} of FA; Barre, 2001) than GO. Several studies revealed almost constant FA composition of these oils regardless the plant variety (Beveridge et al., 2005; Helbig, 2008). The major component of the analyzed oils was linoleic acid ranging from 676 to 732 g · kg^{-1} of FA.

Ruminants feed provides 3% to 6% of FA to the rumen. Forages and concentrate differ in FA composition. Forages tend to be rich in linoleic and α-linolenic acids whereas concentrates are rich in oleic and linoleic acids. Majority of the fat is however hydrolyzed within the rumen, and unsaturated FAs are extensively biohydrogenated prior to their absorption and incorporation into meat and milk fats (Harfoot and Hazlewood, 1988; Cieślak et al., 2001).

FA composition of the diet fed to ruminants may affect the omega-6 to omega-3 ratio as well as the concentration of conjugated linoleic (*cis*9 *trans*11 $C_{18:2}$, CLA) and vaccenic acids (*trans* 11 C18:1, VA) in the rumen, milk and meat (Szumacher-Strabel et al., 2009ab; Cieslak et al., 2010; Szumacher-Strabel et al., 2011a). It has been also shown that some fruit seed oils containing unsaturated FA may mitigate methane production (Szumacher-Strabel et al., 2011b). However, there is no information on the effect of black currant and grape oils, known sources of PUFA, on rumen fermentation, fatty acid composition, methane production and ciliate population. Moreover, chemical composition of diet and interaction among nutrients (e.g. neutral detergent fibre) may modulate the oil action or its efficiency in modifying ruminal fermentation. Such information is crucial when studying oil potential to modulate rumen metabolism. Therefore an experiment was conducted to determine effect of two forages (lucerne and meadow hay) supplemented with GO and BCO plant oils on fermentation parameters, ciliate population and FA content in bovine rumen fluid incubated in batch culture system.

Material and methods

Batch culture fermentation

The rumen inoculum was obtained 3 h after the morning feeding from three rumen-cannulated Polish Holstein-Friesian dairy cows (mean body weight 600 kg) fed with the diet (kg · day^{-1}) containing lucerne silage, 46.0; meadow hay, 1.80; maize meal, 0.90; dry brewer's grains, 0.60; protein concentrate (35% crude protein), 1.50; wheat bran, 0.60; and commercial concentrate (19% crude protein), 5.50.

Ruminal content was squeezed through four layers of cheesecloth into a Schott Duran® bottle (SCHOTT North America, Inc. Corporate Office, Elmsford, NY 10523, USA) with an O_2 – free headspace and immediately transported to the laboratory in a water bath preheated to 39 ± 0.5ºC. Fresh lucerne (300 g · kg^{-1} of dry matter, DM) and wheat meal (600:400, w/w) were used as the components (substrates) of the first diet (LU) for batch culture. Meadow hay (896 g · kg^{-1} of DM) and wheat meal (600:400, w/w) were used as the components (substrates) of a second diet (MH). The substrates (meadow hay, wheat) were ground through a 0.15–0.4 mm screen, bulked and stored in sealed plastic containers. Freshly harvested lucerne was cut into small pieces (0.2–0.4 mm). The substrates (0.78 g lucerne and 0.16 g wheat meal or 0.24 g meadow hay and 0.16 g wheat meal, respectively) were added into each individual batch culture fermentation bottle (100 ml). Both experimental diets (LU and MH) were supplemented with black currant oil (BCO) or grape oil (GO) up to 5% of dry matter of substrates. The oils were extracted from seeds and commercially sold in the province of Wielkopolska, Poland. The oil supplementation doses were established basing on the results of our previous experiment (Cieślak et al., 2006a) on the levels safe for rumen fermentation that is also in agreement with NRC (2001) recommendations. The chemical and FA composition of the diet substrates are presented in Table 1. The *in vitro* experiments were carried out according to Szumacher-Strabel et al. (2004). Briefly, rumen fluid was diluted with a buffer (mg · l^{-1}: K_2HPO_4 292, KH_2PO_4 240, $(NH_4)_2SO_4$ 480, NaCl 480, $MgSO_4 \cdot 7H_2O$ 100, $CaC_2 \cdot 2H_2O$ 64, Na_2CO_3 4, and cysteine HCl 600) in ratio 2:3. Then aliquots of 40 ml were transferred into incubation bottles.

The bottles were filled with CO_2 and then closed with a rubber stopper and aluminum-sealed. In each experiment, the diet was represented by 3 variants

Table 1. Chemical and fatty acid composition of diet ingrediets and FA composition of oil (n = 3)

Components	LU	WM	MH	GO	BCO	SEM
DM, g · kg^{-1}	300	868***	896***	Nd	Nd	3.4
CP, g · kg^{-1} of DM	172	13***	130***	Nd	Nd	0.9
NDF, g · kg^{-1} of DM	196	19***	664***	Nd	Nd	2.9
ADF, g · kg^{-1} of DM	137	4***	330**	Nd	Nd	2.4
ash, g · kg^{-1} of DM	67	2***	56**	Nd	Nd	1.5
Fatty acid, g · kg^{-1}of FA						
C16:0	259	182***	491***	65***	71***	2.5
C16:1 palmitoleic	12	4***	4***	1***	1***	0.5
C18:0 stearic	40	8***	45**	38*	18***	0.9
C18:1*n*-9 oleic	46	149***	82***	177***	132***	1.5
C18:2 linoleic	189	564***	175**	696***	586***	2.4
C18:3 α-linolenic	377	61***	177***	3***	137***	2.7
Total FA, g · kg^{-1}	923	968***	974***	981***	945***	4.8
Other FA, g · kg^{-1}	77	33***	25***	19***	55***	3.3

FA – fatty acids; LU – lucerne, WM – wheat meal, MH – meadow hay, GO – grape oil, BCO – black currant oil, DM – dry matter, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, other FA ($C_{12:0}$, $C_{14:0}$; $C_{20:0}$; $C_{20:1}$; $C_{22:0}$); Nd – not determined
* P < 0.05; ** P < 0.01; *** P < 0.001 express differences from LU

in triplicate: GO, BCO (50 g . kg^{-1} g of DM) and control. The control group comprised of 9 bottles containing all components without oil. Moreover, 9 'blank' bottles with inocula only were included to monitor basal media fermentation activity.

Measurements in batch culture

Chemical analyses of the diet substrates (Table 1) were carried out in triplicates. Dry matter was determined by oven drying at 110ºC for 48 h, whereas neutral (NDF) and acid (ADF) detergent fibres were determined according to method of Van Soest et al. (1991). ADF was expressed including the residual ash. NDF was assayed with sodium sulfite without heat-stable amylase and expressed inclusive of residual ash (Mertens, 2002). Standard methods were used for ash (AOAC Official Method 942.05, AOAC, 1990) and N determination (AOAC Official Method 968.06, AOAC 1990).

All parameters were analyzed after 24 h *in vitro* fermentation. Methane concentration was quantified by gas chromatography in a SRI PeakSimple Model 310 (Alltech, State College, PA, USA) equipped with a thermal conductivity detector (TCD) and Carboxen – 1000 column (mesh side 60/80, 15 FT x 1.8 INS.S, SUPELCO, Bellefonte, PA, USA). Nitrogen was used as the carrier gas at a constant flow of 30.0 ml · min^{-1}. The temperature gradient program was used as follows: initially 180°C for 1.5 min, then increasing by 20°C per min to 220°C. Gas samples of 1 ml were injected. The observed peaks were identified by comparing the retention time swith the appropriate gas standards (the mixture of gas was 5.63% CO_2, 5.56% CH_4, 5.10% H_2, rest N_2, (Multax S.C., Zielonki-Parcele, Poland) using PeakSimple v. 3.29 software (Alltech, State College, PA, USA).

The concentration of volatile fatty acids (VFA, $C_{2:0}$–$C_{4:0}$) after the 24-h fermentation experiments was quantified using liquid chromatography (model 2690, Waters, Santa Clara, CA, USA) according to Czauderna et al. (2008). Analysis of fatty acid methyl esters (FAME) was performed after 24-h fermentation using a VARIAN CHROMAPACK, CP-3380 gas chromatograph (Varian, Inc. Scientific Instruments, Palo Alto, CA, USA) equipped with a flame ionization detector (at 250°C) and a Chrompac CP-Sil 88 column (100 m, 0.25 mm, 0.2 μm film thickness, Varian, Inc. Scientific Instruments, Palo Alto, CA, USA) according to Cieslak et al. (2009a) with some modification. Ultra-high-purity helium was used as the carrier gas at a constant flow of 30.0 ml · min^{-1}. Two μl of each sample were injected in splitless mode. The splitting ratio to the flame ionization detector was 1:90. The oven temperature were programmed as follows: initially 145°C for 9 min, then increasing by 4°C per min to 240°C. FA peaks were identified by comparison with the retention times of known standards (37 FAME Mix, Supelco, Poole, England and $C_{18:2}$ *cis*9 *trans*11, Matreya, Pleasant Gap, PA, USA). Nonadecanoate acid methyl ester ($C_{19:0}$, Sigma-Aldrich, St. Louis, MO, USA) was used as the internal standard. The FA profile was expressed as a percentage of total FA. The FA composition (g · kg^{-1} of FA) of the GO and BCO oil supplements are shown in Table 1. Ammonia was quantified spectrophotometrically using the Nessler reagent, as described by Szumacher-Strabel et al. (2002).

Ciliate counts in batch culture

Samples of the fermentation fluid for counting of ciliate protozoa were collected in duplicates after 24-h fermentation and were fixed with an equal volume of 8% formaldehyde. The ciliated protozoa were counted microscopically according to the procedure described by Coleman (1978). Ciliates were identified according to Dogiel (1927) and Ogimoto and Imai (1981). The following rumen ciliate genera and species were observed: *Entodinium spp.*, *Isotricha spp.*, *Epidinium caudatum*, *Metadinium medium*, *Eudiplodinium maggii*, and *Ostracodinium gracile*.

Calculations and statistical analyses

In vitro dry matter digestibility (IVDMD) was calculated after 24-h incubation using the following equation:

$$\text{IVDMD (\%)} = [(\text{initial DM input} - (\text{Residue} - \text{Blank}) / \text{initial DM input}) \times 100].$$

Statistical analyses were carried out using analysis of variance (Graph Pad Prism; GraphPad Software, San Diego, CA, USA). The data on chemical and FA analyses of the substrates and FA composition of the oils were investigated with one-way analysis of variance using the Newman-Keuls post-test (Table 1). Statistical analyses of measurements were carried out using analysis of variance as a 2 × 3 factorial design representing the two diet groups (lucerne and meadow hay) and three oil supplement subgroups (control without oils, GO and BCO). The effects included in the model were diets (D), oil supplements (O), and interactions between diets and oil supplement (D × O). Differences between controls and the oil additives were analyzed by two-way ANOVA with the Bonferroni post-test. Differences between the treatment means were considered to be significant when $P < 0.05$.

Results

Chemical and fatty acid composition of dietary substrates

All dietary substrates had a different chemical composition (Table 1). The DM of wheat meal and meadow hay was higher ($P < 0.001$), and the CP and ash were lower ($P < 0.001$) compared with lucerne. Compared with lucerne, NDF and ADF ($P < 0.001$) contents were lower in wheat meal and higher in meadow hay ($P < 0.001$). There were also a number of differences in the FA composition of the dietary substrates and oils.

Rumen fermentation parameters *in vitro*

The batch cultures with LU diet were characterized by higher ($P < 0.01$) ammonia N content when compared with the MH diet after 24 h fermentation. The molar proportions of *n*-butyrate and *iso*-valerate were influenced by the diet composition ($P < 0.05$; Table 2). *Iso*-valerate were higher in the LU group whereas *n*-butyrate was higher in the MH group. The diet-oil interaction (D × O) affected the methane production ($P < 0.001$).

Ciliated protozoan population

The effect of diet ($P < 0.001$) on ciliate count was observed (Table 3). Higher number of total ciliates and *Entodinium spp.* and lover number of *Isotricha* spp., *Metadinium medium* and *Eudiplodinium maggii* were observed in LU group when compared with the MH group. Oil supplementation significantly influenced the total ciliate counts and the number of *Entodinium* spp. and *Epidinium caudatum* ($P < 0.05$ and $P < 0.01$) with LU diet. The diet-oil interaction (D × O) affected either total population or ciliate species counts, except for *Isotricha* spp. and *Ostracodinium gracile*.

Fatty acids metabolism

The content of medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and PUFA were influenced by diet composition ($P < 0.05$, $P < 0.01$, and $P < 0.001$) and the oils ($P < 0.001$; Table 4). Concentrations of LCFA, UFA and MUFA were higher for the LU compared with the MH diet. MCFA concentration in oil supplemented samples decreased ($P < 0.01$ and $P < 0.001$ for GO and BCO, respectively) compared with the control, whereas LCFA concentration increased ($P < 0.05$ and $P < 0.01$ for GO and BCO, respectively). The concentrations of UFA ($P < 0.01$), MUFA ($P < 0.05$, $P < 0.01$ for GO and BCO, respectively) and PUFA ($P < 0.01$, $P < 0.001$ for GO and BCO, respectively) were higher in oil supplemented samples when compared with the controls, and the D × O interaction was observed with respect of SFA content ($P < 0.05$).

Except for the *cis* oleic acid concentration (Table 5), diet composition affected the level of all FA ($P < 0.05$, $P < 0.01$ and $P < 0.001$). The VA and α-linolenic acid contents were higher for the LU compared with the MH diet, while linoleic acid, CLA, omega-3 and omega-6 FA were reduced for the LU compared with the MH diet. VA, linoleic acid, CLA, α-linolenic acid and omega-6 FA concentrations were also influenced by oil

Table 2. Effect of grape oil and black currant oil on rumen fermentation parameters after 24-h fermentation in batch culture

Diet	Oil	Methane, mmol · d^{-1}	Ammonia, mmol · l^{-1}	IVDMD, g · kg^{-1} DM	Total VFA, mmol · l^{-1}	Molar proportion of VFA			
						acetate	propionate	*n*-butyrate	*iso*-valerate
	control	4.70	21.6	578	84.1	580	196	133	22.4
LU[1]	GO[1]	3.71	20.1	625	83.0	584	197	131	21.8
	BCO[1]	3.62	21.0	596	88.6	582	202	129	22.1
	control	3.37	16.7	530	83.8	581	190	140	17.5
MH[1]	GO[1]	3.92	14.4	549	83.1	576	188	140	17.6
	BCO[1]	3.75	15.3	582	83.2	573	168	144	17.6
SEM		0.097	1.742	10.2	1.44	2.8	2.9	1.9	0.52
Significance									
Diet (D)		**	**	**	ns	ns	ns	***	***
Oil (O)		*	ns	ns	ns	ns	ns	ns	ns
D × O		***	ns	ns	ns	ns	ns	ns	ns

IVDMD – *in vitro* dry matter degradability, VFA – volatile fatty acids ($C_{2:0}$ – $IC_{4:0}$); [1] see Table 1; ns – not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

Table 3. Effect of grape oil and black currant oil on ciliate population after 24-h fermentation in batch culture

Diet	Oil	Ciliate protozoan population (number · ml^{-1})						
		total ciliate number	*Entodinium* spp.	*Isotricha* spp.	*Epidinium caudatum*	*Metadinium medium*	*Eudiplodinium maggii*	*Ostracodinium gracile*
	control	108200	104800	340	1200	36	3	16
LU[1]	GO[1]	90400	81300	310	1200	29	0	7
	BCO[1]	80600	77500	380	900	51	7	3
	control	76200	70600	450	1400	65	19	1
MH[1]	GO[1]	82600	78200	480	1200	77	27	0
	BCO[1]	81700	78000	480	1300	60	10	0
SEM		3679.3	3128.2	29.3	66.5	7.1	4.1	1.4
Significance								
Diet (D)		***	***	***	***	***	***	ns
Oil (O)		*	**	ns	*	ns	ns	ns
D × O		***	***	ns	*	*	*	ns

[1] see Table 1; ns – not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

Table 4. Composition of total fatty acids (g · kg^{-1} of FA) of rumen fluid incubated with diets supplemented with grape oil and black currant oil after 24-h fermentation

Diet	Oil	Fatty acids (FA)						
		SCFA	MCFA	LCFA	SFA	UFA	MUFA	PUFA
	control	48.1	327	625	735	235	206	59.0
LU[1]	GO[1]	38.0	294	668	701	259	222	76.4
	BCO[1]	37.8	265	697	669	275	234	97.1
	control	47.9	309	643	767	194	164	69.4
MH[1]	GO[1]	17.8	238	743	687	252	209	104
	BCO[1]	18.7	232	749	642	274	225	133
SEM		10.3	8.6	15.3	10.1	8.2	7.4	4.95
Significance								
Diet (D)		ns	***	**	ns	*	**	***
Oil (O)		ns	***	***	***	***	***	***
D × O		ns	ns	ns	*	ns	ns	ns
Control vs. GO		ns	**	*	–	**	*	**
Control vs. BCO		ns	***	**	–	**	**	***

[1] see Table 1; SCFA – short-chain fatty acids ($C_{4:0}$– $C_{13:0}$), MCFA – medium-chain fatty acids ($C_{14:0}$– $C_{17:1}$), LCFA – long-chain fatty acids (>$C_{18:0}$); UFA – unsaturated fatty acids, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, ns – not significant. – D × O is statistically significant, then it is not reasonable to compare the differences between D vs. the control * P < 0.05; ** P < 0.01; *** P < 0.001

Table 5. Composition of fatty acids (g · kg^{-1} of FA) of rumen fluid incubated with diets supplemented with grape oil and black currant oil after 24-h fermentation in batch culture

Diet	Oil	Fatty acids (FA)						
		VA	*cis* oleic	linoleic	CLA	α-linolenic	omega-3	omega-6
LU[1]	control	101	76.5	33.6	1.92	20.9	25.1	28.4
	GO[1]	117	80.0	53.5	3.01	18.6	22.9	37.0
	BCO[1]	135	77.2	70.7	7.32	19.1	25.6	52.0
MH[1]	control	63.5	74.7	44.5	4.88	19.0	25.6	33.9
	GO[1]	107	83.0	84.7	9.25	14.6	32.6	46.2
	BCO[1]	120	86.4	102	11.3	19.0	31.1	75.1
SEM		5.41	4.22	4.57	1.062	0.82	2.85	4.78
Significance								
Diet (D)		***	ns	***	***	*	*	**
Oil (O)		***	ns	***	***	**	ns	***
D × O		ns	ns	ns	ns	ns	ns	ns
Control *vs.* GO		**	ns	**	*	*	ns	*
Control *vs.* BCO		**	ns	***	**	ns	ns	**

[1] see Table 1; VA – *trans*11 $C_{18:1}$, CLA – *cis*9 *trans*11conjugated linoleic acids; ns – not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

treatments (P < 0.01, P < 0.001): VA, linoleic acid, CLA and omega-6 FA contents were higher for both (GO and BCO) oil treatments when compared with the control (P < 0.05, P < 0.01, and P < 0.001), while the α-linolenic acid with GO treatment (P < 0.05) was lower than that of the control. The addition of GO increased the concentrations of PUFA, linoleic acid, *cis*9 *trans*11 CLA and omega-6 FA by 30%, 59%, 57%, and 30%, respectively, with LU diet, and by 50%, 90%, 90%, and 36%, respectively, with MH diet. Supplementing with BCO increased the concentrations of PUFA, linoleic acid, *cis*9 *trans*11 CLA and omega-6 FA by 65%, 110%, 281%, and 83%, respectively, with LU diet, and by 92%, 129%, 132%, and 122%, respectively, with MH diet when compared with control diets.

Discussion

Fermentation parameters, protozoa population and methane production in relation to diet composition and oil supplementation

In the present experiment, two factors were investigated: diet (LU – fresh lucerne and MH – meadow hay) and oil supplementation (black currant BCO and grape GO). Oil supplementation did not affect the basic parameters of rumen fermentation (ammonia, total VFA, and IVDMD). Oils as well as diets influenced methane production, ciliates counts, rumen fluid composition of fatty acids and their isomers. Meale et al. (2012) also obtained changes in methane production when different forage types were evaluated in batch culture system. With regard to the diet composition, ammonia concentration and *in vitro* dry matter digestibility were lower in MH diet. Changes in ammonia concentration may be caused by different content of CP in the analyzed diets (higher in the LU diet). It has been previously reported that increase in the rumen ammonia was associated with bigger populations of ciliates (Veira et al., 1983) what was also observed in the LU diet. In the present experiments, the total VFA and proportion of acetate to propionate were not influenced by diet composition. Diets influenced only the molar proportions of *n*-butyrate and *iso*-valerate. However, these effects were not accompanied by ciliate number increase what may suggest that other factors (e.g. changes in bacterial populations) were involved in the changes of *n*-butyrate and *iso*-valerate. However, it should be underlined that rumen ciliates may produce about 30–46% of VFA (Michałowski, 1987). As far as methane production concerns, the present study revealed a significant reduction only in case of the LU diet supplemented with the two fruit oils (GO and BCO) by 21 and 23%, respectively. Also, other studies showed that oil addition was less effective in case of diets predominated by structural carbohydrates in comparison to non-structural carbohydrates (Cieślak et al., 2006b; Jalč et al., 2006a,b; Machmüller, 2006). The higher content of NDF and ADF in MH diet may create less favourable environment to support the oil action on methane production. Zhang et al. (2008) suggested that suppression of methane production by unsaturated FAs is mediated by their direct action against the rumen microbes involved in methane formation. It should be noted that protozoa are the greatest producers of hydrogen in the rumen ecosystem, and are responsible for up to 37% for methanogenesis in the

rumen. For this reason when population of protozoa is reduced thereby methanogenesis should be also decreased, what was observed in the present study. Moreover, Kišidayová et al. (2006) and Cieślak et al. (2006a, 2009c) suggested that rumen ciliates had no uniform response to oil supplements *in vitro*, what was confirmed also in the current study.

In our experiment, diet composition (LU, MH) exerted more pronounced effect on population of rumen ciliates when considered with the oil supplements. Although protozoa are not essential to rumen fermentation, many of them participate in fiber digestion, and different feed substrates can affect the relative proportion of protozoa and bacteria as well as the extent of rumen fermentation and lipid biohydrogenation products (Michałowski, 1975; Nagadi et al., 2000). Except for *Entodinium* spp., and *Ostracodinium gracile*, the population was composed of large ciliates with high fibrolytic activities, and their numbers increased in the MH diet. This is in accordance with other studies (Michałowski et al., 2001; Béra-Maillet et al., 2005). This suggests that MH diet (containing more NDF and ADF than the LU diet) supplemented with tested oils did not decrease populations of various protozoa.

Diet composition and oil supplementation in relation to FA profile in the rumen fluid

One of the possibilities to enrich ruminant products with beneficial PUFA is the application of high-forage diets (Potkański et al., 2009). The benefits of dietary oils to ruminants are associated with an improvement of bioactive lipid components in ruminant products by alteration of the ruminal microbial population (Mir et al., 2006; Szumacher-Strabel et al., 2011b). In this study, oil supplemented diets incubated for 24 hours in the batch culture system showed an increase in LCFA, UFA, MUFA and PUFA and a decrease in MCFA in the rumen fluid, regardless the diet composition. The observed phenomenon is rather a standard response to supplementation of unsaturated C18 FA (e.g., oleic, linoleic and α-linolenic acid) as reported by Jalč et al. (2007). It should be noted that concentration of FA in the rumen fluid might vary depending on the amount and type of tested oil. In the present experiment, it was also evident that BCO supplementation is more efficient than GO in enhancing PUFA content in rumen fluid. PUFA concentration was also higher in the diet containing meadow hay than lucerne. It is known that PUFA from dried grass are less accessible to the ruminal microbes than those from fresh forage. Besides, dried grass contains PUFA in the form of glycolipids, making them less susceptible to rumen hydrolysis and biohydrogenation (Wachira et al., 2000) and these two processes result in formation of FA isomers like CLA or VA (Or-Rashid et al., 2007; Cieślak et al., 2009b). In the present study, the LU diet contained more VA than the MH diet. By this observation, we showed that diet composition could influence fatty acid profile in the rumen fluid. In addition, oils supplemented to diets also influenced VA content in the rumen fluid, especially when BCO was added, irrespective of the diet used. In animal tissues, VA, an intermediate product formed during LA (linoleic acid) biohydrogenation in the rumen, is used in CLA synthesis by the delta-9-desaturase (Griinari et al., 2000). Diets supplemented with oils rich in LA increased concentration of VA in the rumen fluid (Váradyová et al., 2007; Szumacher-Strabel et al., 2009a). In the present study, GO supplementation decreased α-linolenic acid concentration in both diets, whereas BCO influenced the level of α-linolenic acid only in the LU diet. In our opinion, due to higher content of α-linolenic acid in BCO than in GO, this FA was less biohydrogenated in diets supplemented with BCO. GO was not sufficient source of α-linolenic acid in the MH diet. On the other hand, this contrast was not evident with the LU diet, because fresh lucerne was characterized by a high α-linolenic acid content (377 g . kg^{-1} of FA).

Conclusions

The results of this work indicate that dietary supplementation with grape and black currant oils resulted in higher rumen concentration of PUFA (i.e., linoleic acid, CLA, and omega-6 FA), regardless the diet composition (lucerne or meadow hay). BCO supplementation was found to be more efficient than GO in enhancing PUFA content. Both oils showed the potential to improve the outflow of important FA isomers like VA from the rumen. In relation to diets, lucerne fresh forage provided more favorable rumen environmental conditions expressed e.g. in decrease of methane production and ciliate counts. Further challenges include studies in cattle with different feeding systems to evaluate effects of tested oils on meat and milk composition. These are required before recommending black currant or/and grape oil as animal products modifiers.

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