Effect of microbial oil and fish oil on rumen fermentation and metabolism of fatty acids in artificial rumen

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ABSTRACT: The objective of this study was to examine the effect of microbial oil (MO, n-6 fatty acids) and fish oil (FO, n-3fatty acids) used in their blends as supplements (5% wt/wt) to the diet containing 80% of hay and 20% of barley on rumen fermentation and lipid metabolism in artificial rumen. Overall, three different ratios of n-6 and n-3 fatty acids (1:1, 3:1, and 5:1) as the blends of MO and FO were used. Two similar consecutive experiments were carried out within 2 months. Each experiment lasted for 12 days with 6 days of stabilization period. The addition of all three oil blends did not affect the parameters of fermentation such as degradation of dry matter (DM), detergent fibre, total gas production, but increased the degradation of cellulose and hemicellulose in the diets. The supplementation of oil blends to the diet insignificantly (NS) decreased the methane production (mostly the n-6/n-3 ratio 1:1, about 23.5%), increased ($P < 0.01$) mol% of propionate (mostly the n-6/n-3 ratio 1:1, about 24.1%) and decreased ($P < 0.05$) mol% of acetate (mostly the n-6/n-3 ratio, 1:1, about 7.7%). The lipid metabolism in artificial rumen was also affected, when the oil blends increased ($P < 0.001$) the concentration of total fatty acids (FA) and long-chain FA (LCFA) in effluent. The concentration (mg/g rumen fluid DM) of *trans* (*trans* 11 $C_{18:1}$, TVA-vaccenic acid), *cis* $C_{18:1}$ isomers and CLA-conjugated linoleic acid (*cis* 9, *trans* 11 $C_{18:2}$) was also increased ($P < 0.001$) by the oil blends. Finally, the oil blends caused the incomplete FA biohydrogenation by an increase in TVA concentration and TVA/$C_{18:0}$ ratio in effluent in artificial rumen.

Keywords: microbial oil; fish oil; rumen fermentation; lipid metabolism

Fat supplements are included in the diet of ruminants to increase energy density, improve nutrient utilization, enhance milk and meat yields and affect fatty acid composition (Bauman et al., 2003). The type of diet fed to ruminants influences rumen fermentation and fatty acid profile formed during biohydrogenation (BH). Ruminant diets are usually composed of plants that are rich in polyunsaturated fatty acids (PUFA), e.g. linoleic acid (LA, $C_{18:2}$) and α-linolenic acid (LNA, $C_{18:3}$). Fish oil supplements

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to the ration as an additional source of energy introduce the long-chain fatty acids, EPA (eicosapentaenoic acid, $C_{20:5}$) and DHA (docosahexaenoic acid, $C_{22:6}$) as the predominant FA. Microbial oil isolated from oleaginous microorganisms is another source of various polyunsaturated fatty acids including gamma-linolenic acid (GLA, $C_{18:3}$), dihomo-gamma-linolenic acid (DGLA, $C_{20:3}$), arachidonic acid (AA, $C_{20:4}$) as well as EPA and DHA (Čertík and Shimizu, 1999; Papanikolaou et al., 2004). Microbial oil together with monensin and fumarate was used also in our previous experiments (Jalč and Čertík, 2005). PUFA can be divided into two categories according to the occurrence of double bonds in the fatty acyl chain: n-3 (omega-3) and n-6 (omega-6) fatty acids. Omega-3 FA's include LNA, EPA, and DHA. Omega-6 FA's include LA, GLA, DGLA and AA. It is known that dietary lipids undergo two important transformations in the rumen of ruminants. Rumen metabolism of dietary FA is initiated by microbial lipolysis and subsequent biohydrogenation of free PUFA. During this process, the concentrations of C_{18} PUFA, such as LNA and LA, decrease as they are biohydrogenated completely to stearic acid ($C_{18:0}$) with the formation of intermediates like CLA (*cis* 9, *trans* 11 $C_{18:2}$) and TVA (*trans* 11 $C_{18:1}$) as the most important known ones (Hartfoot and Hazlewood, 1997). Amounts of biohydrogenation intermediates produced in the rumen influence their concentrations in tissues or milk (Loor et al., 2003). TVA and CLA in meat and milk are examples of hydrogenation intermediates that may have beneficial implications in human health. The n-6 and n-3 ratio of fatty acids is highly influenced by the fatty acid composition of the diet fed to the ruminants and affects the concentration of CLA and TVA in the rumen, milk and meat. It is widely accepted that the ideal intake of omega-6 FA's should not exceed the intake of omega-3 FA's more than 4–5 times (Raes et al., 2004). A continuous culture fermenter (artificial rumen) was used to characterize the effect of microbial oil and fish oil blends as the supplements to the diet containing 80% of hay and 20% of barley: (a) on rumen fermentation (degradation of dry matter and detergent fibre, methane production, volatile fatty acid production), (b) on fatty acid metabolism (outputs of *cis* and *trans* isomers of $C_{18:1}$ and $C_{18:2}$ – CLA, TVA during incubation) in this study. Different ratios of n-6 and n-3 FA's (5:1, 3:1, and 1:1) prepared as the mixtures of selected microbial oil and fish oil were used.

MATERIAL AND METHODS

Oil supplements

Microbial oil was isolated from the lower fungus *Thamnidium elegans* CCF 1465 (Culture Collection of Fungi, Department of Botany, Charles University, Prague, Czech Republic). The fungus was maintained on modified Czapek-Dox agar slants with yeast extract (2.5 g/l) at 4°C. The pre-cultivation flasks (100 ml) containing 30 ml cultivation medium (glucose, 30 g/l, corn step, 10 g/l) were inoculated with the fungal spore suspension in an isotonic solution at a final concentration of $1–2 \times 10^6$ spores per ml. After 2 days of pre-incubation on a rotary shaker (reciprocal speed of 130 rpm) at 25°C, 60 ml of pre-incubated culture was transferred to 2 000 ml Erlenmeyer flasks (equipped with buffles to improve aeration) containing 1 000 ml of cultivation medium. The culture was incubated on a rotary shaker (120 rpm) for 5 days at 25°C. After fermentation the mycelium was harvested by filtration, washed with water and gently dried at 65°C for 10 h. Dry fungal biomass was crushed mechanically and total microbial lipid was extracted with hexane with Soxhlet apparatus for 2 h (Čertík and Horenitzký, 1999). Hexane was finally evaporated under vacuum and microbial oil was used for further studies. Fish oil as cod liver oil was obtained from commercial sources. The fatty acid composition (expressed as % of fatty acid methyl esters – FAME) of feed ingredients, meadow hay, barley, MO and FO, is presented in Table 1. The oils were used as blends of MO and FO and the total 5% supplementation of the oil blends was applied in this experiment. Overall, three different ratios of n-6 and n-3 FA (5:1, 3:1, and 1:1) as blends of MO and FO were used.

In vitro fermentation system

The study was carried out using an artificial rumen as described by Czerkawski and Breckenridge (1977). The complete unit composed of four vessels (V_1, V_2, V_3 and V_4), each 850 ml in volume. The general incubation period was described by Czerkawski and Breckenridge (1977). The vessel inoculum was obtained from three ruminally cannulated Slovak Merino sheep (mean body weight 42 ± 2.1 kg) fed 960 g of dry matter (DM) of meadow hay and 240 g DM of crushed barley

Table 1. The fatty acid composition (%) of feed ingredients

(%)	Meadow hay	Barley	Microbial oil	Fish oil
$C_{10:0}$	0.20	–	–	–
$C_{12:0}$	0.60	–	–	–
$C_{14:0}$	1.20	0.30	0.60	4.70
$C_{15:0}$	0.40	0.10	0.10	0.50
$C_{16:0}$	21.0	18.0	16.10	12.40
$C_{16:1}$	2.40	0.20	0.80	6.40
$C_{17:0}$	0.40	0.10	–	0.30
$C_{18:0}$	2.50	1.70	7.20	2.50
$C_{18:1}$, *cis* 9	8.40	16.50	50.20	16.30
$C_{18:1}$, *cis* 7	0.50	0.70	–	–
$C_{18:1}$, *cis* 11	–	–	0.40	3.10
$C_{18:1}$, *trans* 9	–	–	0.10	–
$C_{18:1}$, *trans* 11	–	–	–	0.90
$C_{18:2}$	20.30	54.50	12.60	5.30
$C_{18:3}$ (LNA)	27.70	5.00	–	1.60
$C_{18:3}$ (GLA)	–	–	7.80	–
$C_{20:0}$	1.20	0.20	0.30	–
$C_{20:1}$	–	–	–	8.20
$C_{20:5}$	–	–	–	6.80
$C_{22:5}$	–	–	–	2.90
$C_{22:6}$	–	–	–	9.60
$C_{22:0}$	3.90	0.40	0.50	–
$C_{23:0}$	0.60	0.10	0.10	–
$C_{24:0}$	1.70	0.20	1.40	–
Others	7.00	2.00	1.80	18.30

(%) FAME, fatty acid methyl esters; LNA, α linolenic acid; GLA, γ linolenic acid

in two equal meals. The chemical composition of meadow hay and barley was as follows: DM, 93.44 (89.69); nitrogen, 1.17 (2.18); ash, 9.47 (3.69); neutral detergent fibre (NDF), 58.27 (26.32); acid detergent fibre (ADF), 37.19 (6.79); hemicellulose, 21.07 (19.49); cellulose, 29.50 (5.41); lignin, 7.68 (7.72) as % of initial DM. Fermentation inocula (solid and liquid ones) were collected through the rumen cannula immediately before the morning feeding and transferred to the artificial rumen. The solid digesta (80–100 g of wet weight) were placed into nylon bags (100 μm pore size) in each of the four fermentation vessels. The vessels were filled to overflowing with strained rumen fluid and artificial saliva (1:1) (McDougall, 1948). Including the first day of the experiment, the vessels were supplied with 12.8 g (11.96 g DM) of meadow hay and 3.2 g (2.87 g DM) of barley at the daily intervals. Vessel V_2 received also 5% (wt/wt) of the oil blends (MO + FO) with the n-6 to n-3 FA ratio 1:1; V_3 received 5% (wt/wt) of the oil blends (MO + FO) with the n-6 to n-3 FA ratio 3:1 and V_4

received 5% (wt/wt) of the oil blends (MO + FO) with the n-6 to n-3 FA ratio 5:1. Vessel V_1 was the control (without oil addition). To ensure that all vessels contained 12% crude protein (CP), 223 mg of urea were added in 1 000 ml McDougall buffer. A continual infusion of artificial saliva (pH 8.4) at the rate 665–728 ml was maintained through each vessel during the experiment.

Measurements and chemical analyses

Two similar consecutive experiments were carried out in artificial rumen within 2 months. Each experiment in artificial rumen lasted 12 days. To ensure a steady state within the vessels a 6-day adaptation period was followed by a 6-day collection period. On days 6–12 the following samples were collected: produced gas was collected into special bags and volumes of gas were measured with a gas-meter and methane concentrations were analysed in a gas chromatograph (Perkin-Elmer, Clarus 500) as reported by Czerkawski and Clapperton (1968). Liquid effluent was collected into flasks placed in ice bath and samples were taken for volatile fatty acids (VFA), ammonia nitrogen (NH_3-N) and fatty acid (FA) analyses. The daily productions of VFA were analysed by the gas chromatography procedure (Cottyn and Boucque, 1968). Ammonia nitrogen concentrations were measured by a microdiffusion method (Conway, 1962). The fatty acid content in effluent was determined in lyophilized samples. Lipids were extracted from 500 mg of freeze-dried effluent, meadow hay and barley using chloroform and methanol (2:1, vol/vol) followed by 6N HCL as described by Fellner et al. (1995). Heptadecenoate $C_{17:0}$ (Supelco, USA) was used as the internal standard. Fatty acids of total lipids were analysed as their methyl esters (Christoperson and Glass, 1969) by gas chromatography according to Čertík et al. (2005). Dry matter, ash and nitrogen were analysed according to the methods of the Association of Official Analytical Chemists (AOAC, 1980). Neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose in feed and residual feed samples were analysed by the method of Goering and Van Soest (1970). The other fermentation variables, energetic efficiency of VFA (Ørskov et al., 1968) and OMF, organic matter fermented (Demeyer and Van Nevel, 1979), were calculated from the stoichiometry of rumen fermentation.

Statistical analysis

Means of results from treatments were compared by one-way analysis of variance (Graphpad InStat, Graphpad Software Inc., San Diego, CA USA). Treatment means were statistically compared by the Turkey-Kramer multiple comparison test. The tables give the group means and the standard error of the mean (± SEM). Probability values of $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Effect of modifying n-6/n-3 ratio of dietary oil supplements on rumen fermentation *in vitro*

Rumen metabolism can be characterized by a fermentation pattern, consisting of the following parameters: the amount of molar proportions of volatile fatty acids produced; the amount of methane formed and OMF (Demeyer and Van Nevel, 1986). All these parameters of rumen fermentation were determined in this study. The n-6/n-3 ratio is highly influenced by the fatty acid composition of the diet fed to the animals. Finishing ruminants on pasture can decrease the n-6/n-3 ratio to a value of 2 or less, while concentrate fed ruminants had the ratios around 6–10 (Raes et al., 2004). In our study, the effect of modifying the n-6/n-3 ratios of dietary oil supplements to the diet consisting of hay and barley (80:20%) on the parameters of rumen fermentation in artificial rumen was studied. Fish oil contained higher amounts of EPA (6.8% FAME) and DHA (9.56% FAME) and lower amounts of LNA (1.63% FAME) as the sources of n-3 FA (Table 1). Microbial oil contained higher amounts of LA (12.63% FAME) and lower amounts of GLA (7.83% FAME) as the sources of n-6 FA (Table 1). Microbial oil is an alternative source of animal and plant oils (Čertík and Shimizu, 1999; Ratledge, 2003). In our study, the addition of oil blends up to 5% in DM at different ratios of n-6/n-3 FA to hay-barley diet showed a slight effect on some parameters of rumen fermentation *in vitro* (Table 2). The degradation of DM, NDF, ADF was not affected by the supplementation of oil blends. We could only determine the higher ($P < 0.05$) values of hemicellulose in fermentation vessels V_2, V_3, V_4, and cellulose in V_2 and V_4. Some papers reported no negative effects of supplemental saturated or unsaturated fat (Schroeder

Table 2. The effect of microbial and fish oil blends as supplements in the diet containing hay and barley (80:20%) on rumen fermentation in artificial rumen (n-12)

Fermentation vessel	Control	MO + FO blend	MO + FO blend	MO + FO blend
	V_1	V_2	V_3	V_4
n-6:n-3 ratio	–	1:1	3:1	5:1
DMD (%)	49.30 ± 1.0	46.90 ± 0.9	48.90 ± 1.7	48.80 ± 0.7
NDF (%)	18.70 ± 1.7	19.70 ± 1.3	23.80 ± 2.6	25.10 ± 1.0*
ADF (%)	19.90 ± 1.7	18.70 ± 1.4	25.30 ± 2.5	19.50 ± 1.2
Hemicelluloses (%)	16.30 ± 1.7	20.80 ± 1.3*	21.40 ± 2.7*	33.20 ± 0.9***
Cellulose (%)	29.30 ± 1.4	38.70 ± 1.1*	34.90 ± 2.2	41.20 ± 0.8**
VFA (mmol/day)	36.20 ± 0.9	35.50 ± 1.0	37.90 ± 1.0	36.60 ± 0.9
Acetate (mol%)	59.70 ± 0.2	55.10 ± 0.2*	58.0 ± 0.3*	59.0 ± 0.2*
Propionate (mol%)	19.50 ± 0.2	24.20 ± 0.3**	21.20 ± 0.3*	21.20 ± 0.3*
n-butyrate (mol%)	14.90 ± 0.3	14.60 ± 0.3	15.10 ± 0.4	14.80 ± 0.4
Total gas (l/day)	3.30 ± 0.1	3.20 ± 0.1	3.20 ± 0.1	3.40 ± 0.1
Methane (mol/day)	6.80 ± 0.4	5.20 ± 0.5	6.40 ± 0.3	6.80 ± 0.3
NH_3-N (mg/100ml)	12.40 ± 0.6	14.40 ± 1.0	18.30 ± 1.0**	17.10 ± 1.2*
E (%)	74.90 ± 0.1	76.90 ± 0.1**	75.70 ± 0.1*	75.50 ± 0.1*
OMF (g/day)	6.70 ± 0.1	6.30 ± 0.1	6.60 ± 0.1	6.60 ± 0.1

MO = microbial oil; FO = fish oil; DMD = dry matter digestibility; E = energetic efficiency of VFA's; OMF = organic matter fermented; ±SEM = standard error of the mean; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ differences from control

et al., 2002) or linseed, soybean and cottonseed oil supplements (4% wt/wt) with the dietary n-6/n-3 fatty acid ratio of 2.3:1, 8.8:1, 12.8:1 and 15.6:1, respectively, on apparent digestibility of DM, NDF or ADF (Kim et al., 2007). On the contrary, Choi et al. (1998) reported a non-significant trend towards on increase in fibre digestion in the rumen with the addition of fish oil. Our experiments showed that total gas production (3.2–3.3 l/day) was similar in all fermentation vessels. The methane production (mmol/day) was numerically (NS) reduced in V_2 (about 23.5%) and in V_3 (about 5.9%). There were not any different values in the methane production in V_4 compared to the control (Table 2). Our previous *in vitro* study with MO, borage oil (BO) and evening primrose oil (EPO) supplementation (5% wt/wt) of the diet consisting of hay and barley (60:40%) showed numerically decreased methane production by about 11.3% (MO), 2.04% (BO) and 11.4% (EPO, Jalč et al., 2005). Methane suppression with oil blend supplementation was accompanied by a shift of the fermentation pattern towards propionate without any effect on total VFA production (Table 2). Molar proportions of propionate were increased ($P < 0.01$) in V_2 (about 4.7 units), in V_3 and V_4 ($P < 0.05$; about 1.7 units). Molar proportions of acetate were decreased ($P < 0.05$) in V_2 (about 4.6 units), in V_3 and V_4 ($P < 0.05$; about 0.7 to 1.7 units), while molar proportions of n-butyrate were not affected by dietary treatment with the oil blend (Table 2). Kim et al. (2005) found that the oil supplementation containing the n-6/n-3 ratios of 2:1, 10:1, 16:1, and 20:1 by mixing linseed oil, cottonseed oil and soybean oil did not affect the ruminal concentrations of acetate and propionate in lambs. The NH_3-N pool produced by degradation of urea in artificial saliva (McDougall buffer) and feed nitrogen are the main sources of nitrogen used by bacteria for protein synthesis. Ammonia nitrogen concentrations depend on ammonia use and release by the microbial population (Mansfield et al., 1995). The results showed that the supplementation of oil blends with the n-6/n-3 ratio 3:1 and 5:1 in V_3 ($P < 0.01$) and V_4 ($P < 0.05$)

Table 3. The effect of microbial and fish oil blends as supplements in the diet containing hay and barley (80:20%) on lipid metabolism in artificial rumen (n-12)

Fatty acid, (mg/g) rumen fluid DM	Control	MO + FO	MO + FO	MO + FO	Pooled SEM
Vessel	V_1	V_2	V_3	V_4	
n-6/n-3		1:1	3:1	5:1	
$C_{14:0}$	0.11	0.35***	0.38***	0.26**	0.02
$C_{15:0}$	0.24	0.45***	0.36	0.25	0.02
$C_{16:0}$	1.03	2.36***	2.32***	2.14***	0.05
$C_{16:1}$, *cis* 9	0.22	0.55***	0.58***	0.54***	0.02
$C_{17:0}$	5.21	5.06	5.25	5.22	0.13
$C_{18:0}$	0.82	0.62*	0.83	0.94	0.06
TVA	0.15	0.69***	0.78***	0.82***	0.01
$C_{18:1}$, *cis* 9	0.22	0.86***	0.88***	0.67***	0.02
$C_{18:1}$, *cis* 11	0.13	0.67***	0.69***	0.82***	0.03
$C_{18:2}$, *cis* 9, 12	0.21	0.22	0.18	0.17	0.01
CLA	0.13	0.24***	0.26***	0.32***	0.01
Total FA	8.22	11.20***	11.60***	11.50***	0.22
MCFA (%)	83.20	75.70*	73.80**	71.90***	2.12
LCFA (%)	16.80	24.30***	26.10***	28.0***	1.40
SFA (%)	90.50	76.0***	75.80***	75.20***	2.10
UFA (%)	9.40	30.70***	29.20***	32.10***	0.60
SFA/UFA	9.60	2.50***	2.60***	2.30***	0.50

MCFA = medium-chain fatty acids ($C_{14:0}$–$C_{17:0}$); LCFA = long-chain fatty acids > $C_{18:0}$; SFA = saturated fatty acids; UFA = unsaturated fatty acids; TVA, *trans* 11 $C_{18:1}$; CLA, *cis* 9, *trans* 11 $C_{18:2}$; *P < 0.05; **P < 0.01; ***P < 0.001 differences from control

increased the NH_3-N concentration in effluent, while its concentration in V_2 was not affected by the supplementation of oil blends with the n-6/n-3 ratio 1:1 (Table 2). In the other experiment, the supplementation of oil blends (5% wt/wt) – linseed oil (LO), rapeseed oil (RO) and FO as LO + RO, LO + FO, and LO + RO + FO to the diet consisting of 60% fresh lucerne and 40% maize caused an increase in the ammonia nitrogen concentration in effluent (Jalč et al., 2006). The energetic efficiency of VFA (E) was increased (P < 0.01–0.05) by the oil blends, mainly by the oil blends of the n-6/n-3 ratio 1:1. This was evoked by an increase in mol% of propionate in the diets after the oil blend treatment. The amount of OMF was unchanged by the oil blend supplementation to the mixed diet in artificial rumen.

Effect of modifying n-6/n-3 ratio of dietary oil supplements on lipid metabolism *in vitro*

The lipid composition of meadow hay mostly consists of glycolipids and phospholipids, and major unsaturated fatty acids are LNA and LA (Table 1). The lipid composition of barley consists of triglycerides and major FA's are oleic acid ($C_{18:1}$) and LA (Table 1). The studied microbial oil was rich in $C_{18:1}$ and GLA, and fish oil contained higher levels of EPA and DHA (Table 1). Therefore, the elevated

ratio of n-6/n-3 FA's in the oil mixtures (1:1 > 3:1 > 5:1) was a consequence of increased amounts of MO to FO used for the oil mixtures. The rumen microbial lipid metabolism is characterized by lipolysis of dietary glycolipids, phospholipids and triglycerides and leads to a release of free fatty acids. The formed free FA's are hydrogenated by rumen microbes to saturated end products such as stearic acid with the formation of intermediates like CLA and TVA (Hartfoot and Hazlewood, 1997). Overall, the addition of oil blends (n-6/n-3 1:1, 3:1, 5:1) to the feed resulted in the increased total fatty acid concentration in rumen fluid effluent (mg/g rumen fluid DM) by 36%, 41%, and 40%, respectively (Table 3). An increase in the total fatty acid concentration in rumen fluid is a typical response to the lipid supplementation of ruminant diets (Beaulieu et al., 2002). The supplementation of oil blends to the diet significantly decreased the percent proportion (%) of MCFA ($C_{14:0}$ – $C_{17:0,}$ about 7.4–11.2%) and increased ($P < 0.001$) the amount of LCFA (> $C_{18:0}$, about 7.5–11.6%) in effluent. The degree of BH of individual fatty acids was not estimated in this study; however many authors have reported different rates of BH of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ FA. Wachira et al. (2000) reported that BH of $C_{18:2}$ and $C_{18:3}$ ranged between 80 and 93% when lambs were fed fish oil. Biohydrogenation of EPA and DHA increased from 49 and 74% to 79 and 86%, respectively, when fish oil in the concentrate increased from 1 to 4 g per 100 g (Lee et al., 2005). It is known that BH of PUFA is characterized by: (a) changes in the percent proportions of unsaturated (UFA) and saturated (SFA) fatty acids in the rumen, (b) accumulation of *trans* fatty acids in the rumen, (c) higher concentration of stearic acid (AbuGhazaleh et al., 2002). We can state that the extent of BH of unsaturated FA in our experiment was affected by the oil blends and was characterized by: (a) accumulation of *trans* fatty acids, especially TVA in effluent, (b) changes in % proportion of SFA and UFA in effluent. Indeed, UFA concentration (%) increased ($P < 0.001$; about 20–23%, mainly the n-6/n-3 ratio 3:1) and SFA concentration (%) decreased ($P < 0.001$; about 14–15%, mainly the n-6/n-3 ratio 3:1) when oil blends were supplied in comparison with the control. Thus, the diet enriched with oil mixtures resulted in a decrease ($P < 0.001$) in the ratio of SFA/UFA (about 4 times) compared to the control. However, the concentration of stearic acid was slightly ($P < 0.05$) or not significantly decreased in the diets supplemented with oil mixtures (n-6/n-3 ratio, 1:1, 3:1) or slightly (NS) increased with oil blends (n-6/n-3, ratio 5:1). AbuGhazaleh et al. (2002) also found a lower proportion of $C_{18:0}$ in ruminal digesta when 2% menhaden oil or 1% menhaden oil plus 1% extruded soybeans were added to TMR (total mixed ration) for cows. The accumulation of *trans* intermediates is probably due to an excess of free fatty acids, which inhibits the final hydrogenation of $C_{18:1}$ *trans* isomers to stearic acid (Gulati et al., 2000). In our experiment, the concentration of the main *trans* $C_{18:1}$ isomer – TVA in effluent increased ($P < 0.001$; 4.6–5.2 times) with all three oil blends. This increase in TVA may be caused by the inhibition of reductase activity of ruminal microbes with DHA and EPA present in fish oil. AbuGhazaleh and Jenkins (2004) observed that the addition of DHA, soybean oil or their mix to ruminal cultures *in vitro* increased *trans* $C_{18:1}$ isomers by 141, 100 and 266%, respectively, compared with the control. The concentration of *cis* $C_{18:1}$ isomers (*cis* 9 and *cis* 11) increased ($P < 0.001$) in diets supplemented with oil blends 3.9 or 5.1; 4 or 5.3; and 3 or 6.3 times (n-6/n-3 ratio, 1:1, 3:1, 5:1). Mosley et al. (2002) found that *cis* 9 $C_{18:1}$ isomer could serve as a precursor for several *trans* FA isomers. The *cis* 9 $C_{18:1}$ might also interfere with BH of other PUFA in the diet, resulting in the accumulation of *trans* $C_{18:1}$ isomers. The increase in TVA concentration and in TVA/$C_{18:0}$ ratio from 0.18 (control) to 1.11, 0.94, 0.87 (n-6/n-3 ratio, 1:1, 3:1, 5:1), respectively, is an indication of the incomplete biohydrogenation of unsaturated FA with fat supplements (Table 3). The concentration of the other main isomer (CLA, *cis* 9, *trans* 11 $C_{18:2}$) increased ($P < 0.001$) as the n-6/n-3 ratio increased (Table 3). Similar results were reported by Váradyová et al. (2007), who studied the effect of sunflower oil and rapeseed oil (5% wt/wt) supplement to meadow hay-barley grain (80:20%) diet in sheep on the profile of fatty acids and their isomers (CLA, TVA) in rumen fluid. On the contrary, Kim et al. (2005) found that the CLA concentration decreased as the n-6/n-3 ratio increased. These authors investigated the effect of modifying the n-6/n-3 ratio of dietary oil supplement treatments of 2:1, 10:1, 16:1 and 20:1 by mixing linseed oil, cottonseed oil and soybean oil in lambs.

It can be stated that the supplementation of oil blends with the n-6/n-3 ratio 1:1, 3:1, 5:1 to a mixed diet (hay-barley, 80:20%): (a) increased the degradation of hemicellulose and cellulose in the diets; (b) numerically (NS) decreased the methane pro-

duction (mostly the n-6/n-3 ratio 1:1, about 23.5%), increased ($P < 0.01$) mol% of propionate (mostly the n-6/n-3 ratio 1:1, about 4.7 units) and decreased ($P < 0.05$) mol% of acetate (mostly the n-6/n-3 ratio 1:1, about 4.6 units); (c) increased ($P < 0.001$) the concentration of total FA and % proportion of LCFA in effluent; (d) increased ($P < 0.001$) the production of *trans* (*trans* 11 $C_{18:1}$, TVA) and *cis* (*cis* 9, *cis* 11) $C_{18:1}$ isomers; (e) increased ($P < 0.001$) the production of *cis* 9, *trans* 11 $C_{18:2}$ (CLA). Finally, the oil mixture supplementation caused the incomplete biohydrogenation of fatty acids characterized by an increase in TVA concentration and TVA/$C_{18:0}$ ratio in effluent.

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