Effect of microbial oil, evening primrose oil and borage oil on rumen fermentation *in vitro*

D. Jalc[1], A. Potkanski[2], M. Szumacher-Strabel[2], A. Cieslak[2], M. Certik[3]

[1]Institute of Animal Physiology, Slovak Academy of Sciences, Kosice, Slovak Republic
[2]August Cieszkowski Agricultural University, Poznan, Poland
[3]Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovak Republic

ABSTRACT: The objective of this study was to examine the effects of microbial oil, evening primrose oil and borage oil on rumen fermentation of a diet consisting of 80% of hay and 20% of barley in an artificial rumen (Rusitec). All three oils contained gamma-linolenic acid (GLA), microbial oil – 8.4%, evening primrose oil – 9.2% and borage oil – 23.7% out of the total fatty acid content. The experiment in Rusitec lasted 11 days. After a stabilization period (5 days), microbial oil (5% wt/wt) was added into fermentation vessel V_2, evening primrose oil (5% wt/wt) into V_3 and borage oil (5%wt/wt) into V_4 (6 days). Fermentation vessel V_1 served as a control (without oils). The results showed that the oils did not affect any of the basal parameters of rumen fermentation (pH, NH_3-N, degradation of dry matter, organic matter, neutral detergent fibre, acid detergent fibre). Methane production (mmol/day) was reduced numerically by the oils; microbial oil, evening primrose oil and borage oil decreased CH_4 production about 11.32%, 11.45% and 2.04%, respectively. The supplementation of the oils to the total mixed ration (TMR) significantly decreased percentage proportions of short-chain fatty acids (SCFA, about 0.1–0.3%), medium-chain fatty acids (MCFA, about 8%) and increased long-chain fatty acids (LCFA, about 8%) in the effluent. Stearic acid $C_{18:0}$ was the major FA in the effluent and was significantly reduced in oil supplemented diets. The percentage proportion of *trans* $C_{18:1}$ isomers significantly increased (1.7–2 times) in all oil supplemented diets. The main intermediates – *cis* 9, *trans* 11 $C_{18:2}$ (CLA) and trans 11 $C_{18:1}$ (TVA) also increased after oil supplementation of the diet. TVA concentration with microbial oil, evening primrose oil and borage oil supplementation was 3.17%, 8.19% and 9.3% in comparison with the control (1.38%). CLA concentration significantly increased 2.3, 1.2, and 2.1 times after microbial oil, evening primrose oil and borage oil supplementation in Rusitec. Finally, the oil supplementation caused incomplete biohydrogenation of unsaturated FA and it was characterized by an increase in TVA concentration and TVA to $C_{18:0}$ ratio in oil supplemented diets.

Keywords: microbial oil; evening primrose oil; borage oil; rumen fermentation; CLA; TVA

Evening primrose oil, borage oil and microbial oil have been getting increasing attention for their potential in human medicine (Barre, 2001). Evening primrose oil is derived from the seed of *Oenothera biennis*. It contains about 9% (9 g/100 g of total fatty acids /FA/) of GLA – gamma linolenic acid ($C_{18:3}$, n-6). Borage oil is derived from the seed of *Borago officinalis*. It contains about 23% GLA out of total FA. Microbial oil is produced by the fungus *Thamnidium elegans*. This fungus grows on a mixture of wheat bran/spent malt grains (3 : 1). Microbial oil contains about 8.4% of GLA out of total FA. GLA is an intermediate in the conversion of linoleic acid – LA ($C_{18:2}$, n-6) to arachidonic acid – AA ($C_{20:4}$, n-6) (Horrobin, 1990).These polyunsaturated fatty acids (PUFA) serve as precursors of a

Supported by the Grant Agency of Science of the Slovak Academy of Sciences – VEGA (Grants No. 2/3058/25 and No. 2/6174/6).

wide variety of metabolites (such as prostaglandins, leukotrienes and hydroxyl fatty acids) regulating critical biological functions (Certik and Shimizu, 1999). PUFA deficiencies lead to abnormalities in the skin, in the nervous system, immune and inflammatory systems, cardiovascular system, endocrine, respiratory and reproductive systems. Because the mammals lack the ability to synthesize PUFA, they must be supplied in a diet. Evening primrose oil is applied to human patients with the following disorders – primary (diabetes, eczema), secondary – osteoporosis (in combination with fish oil), alcohol withdrawal, rheumatoid arthritis, phenylketonuria, others – atherosclerosis, scleroderma, etc. Borage oil is applied to human patients with the following disorders – primary (rheumatoid arthritis), other – infantile seborrhoeic dermatitis. No information is available about the use of evening primrose oil, borage oil and microbial oil in ruminants where the effects of these oils on rumen fermentation *in vitro* were observed. The effect of substitution of evening primrose oil cake for soybean oil meal was investigated in calves (Strzetelski et al., 1998a) and the effect of addition of evening primrose oil cake on milk fatty acid composition, animal performance, hormone and metabolite levels in blood plasma was studied in cows (Strzetelski et al., 1998b). The rumen degradation characteristics of borage meal were also studied when borage meal supplemented diets were fed to growing lambs (Mustafa et al., 1997) and the effect of diet containing evening primrose oil on the content of functional lipid fractions in goat milk was investigated (Reklewska et al., 2002).

The aim of this paper was to study: (a) the effect of evening primrose oil, borage oil and microbial oil as supplements (5% wt/wt) of a diet containing 60% hay and 40% barley on the rumen fermentation *in vitro*; (b) the effect of evening primrose oil, borage oil and microbial oil on trans fatty acid production (mainly *cis* 9, *trans* 11 $C_{18:2}$ – CLA and *trans* 11 $C_{18:1}$ – TVA) in the fermentation fluid in a semicontinuous fermenter (Rusitec).

MATERIAL AND METHODS

Fat supplements

Microbial oil was produced by the fungus *Thamnidium elegans*. The fungus was grown on a mixture of wheat bran/spent malt grains (3 : 1). Microbial oil was supplied by the Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic. Borage oil and evening primrose oil were obtained from commercial sources and also from the same source as microbial oil. The fatty acid composition (mg/g of fatty acids) all three oils is presented in Table 1.

In vitro fermentation system

The experiment was carried out using the rumen simulation technique (Rusitec) as described by Czerkawski and Breckenridge (1977). The fermentation equipment was composed of four fermentation vessels (V_1, V_2, V_3, 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 44.3 ± 1.8 kg) fed 780 g dry matter (DM) of meadow hay and 520 g DM of barley in two equal meals. The chemical composition of meadow hay and barley was as follows: DM 91.07 (86.91); nitrogen 1.14 (2.11); ash 9.24 (3.58); neutral detergent fibre (NDF) 56.79 (25.21); acid detergent fibre (ADF) 36.25 (6.59); hemicellulose 20.54 (18.91); cellulose 28.76 (5.25); lignin 7.49 (7.49) as percentages of original DM. Fermentation inocula (solid and liquid ones) were collected through the rumen cannula immediately before morning feeding and transferred to an artificial rumen. The solid digesta (80–100 g wet weight) were placed into nylon bags (100μm 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 the diet 9.9 g (9.0 g DM) of meadow hay and 6.9 g (6.0 g DM) of barley at daily intervals. Fermentation vessel V_2 received also 5% (wt/wt) addition of microbial oil (MO), V_3 received 5% (wt/wt) supplementation of evening primrose oil and V_4 received 5% supplementation of borage oil. Fermentation vessel V_1 served as a control (without oil supplementation). To ensure that all vessels contained 12% crude protein (CP), 94.4 mg of urea were added in 1 l of McDougall buffer. A continual infusion of artificial saliva (pH 8.4) at the rate of 665–758 ml was maintained through each vessel during the experiment.

Measurements and chemical analyses

The experiment in Rusitec lasted 11 days. To ensure a steady state within the vessels a 5-day adaptation period followed by a 6-day collection period was used. On days 6–12 the following samples were collected. The samples of effluents, collected into flasks placed in ice bath, were taken for the analyses of fatty acids (FA) and ammonia nitrogen. NH_3-N concentration in the effluent was measured by a microdiffusion method (Conway, 1962). Fatty acids in a fermentation fluid (effluent) were analysed according to the modified method of Czauderma and Kowalczyk (2001). The samples of the effluent (10 ml) were hydrolysed with 3 ml NaOH at 90°C for 40 min and then the hydrolysed samples were acidified with 4M HCl to pH ~2. The free fatty acids were extracted three times with diethylether (2 ml). Fatty acids in extracted lipids were methylated with 0.5N NaOH in methanol and 10% boron trifluoride in methanol. Diundecenoate $C_{11:0}$ was used as the internal standard. The methylated samples were injected in the ion-exchange columns (Ag^+ – HPLC system). A Waters (Milford, MA, USA) HPLC 625 LC system was used. The gas produced was collected into special bags and the gas volumes were measured with gas-meter and methane concentrations were analysed in a Perkin Elmer 8500 gas chromatograph as reported by Czerkawski and Clapperton (1968). Dry matter, ash and nitrogen were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1980). NDF, ADF, cellulose, hemicellulose and lignin in feed and residual feed samples were determined according to Goering and Van Soest (1970).

Statistical analysis

Means of results from treatments were compared with one-way analysis of variance (ANOVA). Treatment means were statistically compared with the Tukey-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.

Table 1. Fatty acid composition of dietary components and fat supplements

Components (mg/g of fatty acids)	Meadow hay	Barley	MO	EPO	BO
$C_{14:0}$	0.29	0.09	7.0	–	–
$C_{15:0}$	0.09	0.03	–	–	–
$C_{16:0}$	5.19	5.86	153.3	50.0	114.0
$C_{16:1}$	0.59	0.07	4.0	–	1.0
$C_{17:0}$	0.10	0.03	–	–	–
$C_{18:0}$	0.61	0.54	101.0	10.0	28.0
$C_{18:1}$	2.12	5.63	509.0	47.0	201.0
$C_{18:2}$	5.0	17.77	139.0	801.0	414.0
$C_{18:3}$ (ALA)	6.83	1.58	–	–	–
$C_{18:3}$ (GLA)	–	0.04	84.0	92.0	237.0
$C_{20:0}$	0.31	0.08	–	–	–
$C_{20:1}$	–	0.05	–	–	–
$C_{22:0}$	0.96	–	–	–	–
$C_{22:1}$	–	0.04	–	–	–
$C_{23:0}$	0.14	–	–	–	–
$C_{24:0}$	0.43	0.05	–	–	–

MO – microbial oil, EPO – evening primrose oil, BO – borage oil, ALA – α-linolenic acid, GLA – γ-linolenic acid

RESULTS AND DISCUSSION

Effect of microbial oil, evening primrose oil, and borage oil on rumen fermentation *in vitro*

In this study, the addition of oil up to 5% in DM as evening primrose oil, borage oil and microbial oil to a hay : barley (80% : 20%) diet did not have any effects on the basal parameters of rumen fermentation *in vitro* such as pH of fermentation fluid and ammonia nitrogen concentration in the effluent (Table 2). Lipid supplementation of diets mostly reduces rumen degradation of fibre as well as of organic matter (Machmuller et al., 1998). In this study, the rumen degradation of dry matter (DM) and organic matter (OM) was similar in all fermentation vessels and was not affected by the type of oil supplementation. The degradation of fibre, NDF and ADF was similar in all fermentation vessels. There were no significant differences between control and oil supplemented diets (Table 2). We recorded only significantly higher values of hemicellulose in V_3 and significantly lower values of hemicellulose in V_2 and V_4. In the literature, Sutton et al. (1983) and Wachira et al. (2000) found out the negative effects of PUFA in the oils on fibre digestion in wethers and beef cattle. On the contrary, Choi et al. (1998) reported a non-significant trend towards an increase in fibre digestion in the rumen with the addition of fish oil. The total gas production (l/day) was similar in all fermentation vessels. The methane production (mmol/day) was numerically reduced about 11.32% with microbial oil, 11.45% with evening primrose oil and 2.04% with borage oil supplementation. With the hay diet, the reduction in CH_4 production induced by canola oil and cod liver oil (10% wt/wt) was 26 and 29%, respectively (Dong et al., 1997) and was similar to CH_4 reductions (27, 26, and 34%) caused by infusing 60 g/day of oleic, linoleic and linolenic acid, respectively, into the rumen of sheep fed dried hay (Czerkawski et al., 1966). Our previous *in vitro* study with linseed, sunflower and rapeseed oil (10% wt/wt) supplementation to the diet consisting of hay, barley and molasses (60 : 30 : 10%) showed a numerical decrease of the methane production about 30, 33, and 30% (Jalc et al., 2002).

Effect of microbial oil, evening primrose oil and borage oil on *trans* fatty acid production in fermentation fluid

The lipid composition of meadow hay consists largely of glycolipids and phospholipids, and the major fatty acids (FA) are the unsaturated fatty

Table 2. Effects of microbial oil, evening primrose oil and borage oil on some parameters of rumen fermentation *in vitro*

	V_1 control – (TMR)[a]	V_2 TMR + MO (5%)[b]	V_3 TMR + EPO (5%)[c]	V_4 TMR + BO (5%)[d]
Degradation of feed after 48 h (%)				
Dry matter	55.8 ± 2.7	52.9 ± 2.0	54.8 ± 1.9	53.3 ± 1.7
Organic matter	55.6 ± 2.7	53.0 ± 2.0	54.5 ± 1.9	53.1 ± 1.7
NDF	27.3 ± 4.5	25.0 ± 3.1	37.2 ± 4.2	25.7 ± 2.7
ADF	17.2 ± 5.1	16.1 ± 3.5	16.6 ± 3.5	26.7 ± 3.2
Hemicellulose	39.4 ± 3.7	34.8 ± 2.8[c]	46.9 ± 2.2[d]	28.2 ± 2.7
Cellulose	26.0 ± 4.6	23.1 ± 3.2	25.5 ± 3.1	26.7 ± 2.8
Lignin	22.5 ± 4.7	27.1 ± 3.0[c]	22.0 ± 3.2[d]	42.0 ± 2.2
pH	7.1 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1
Total gas production (l/day)	3.5 ± 0.2	3.6 ± 0.1	3.5 ± 0.1	3.6 ± 0.1
Methane (mmol/day)	7.3 ± 0.6	6.5 ± 0.3	6.5 ± 0.4	7.2 ± 0.1
NH_3-N (mg/l)	134.1 ± 7.7	137.1 ± 4.0	114.1 ± 6.7	131.1 ± 5.6

TMR – total mixed ration, MO – microbial oil, EPO – evening primrose oil, BO – borage oil, EMS – efficiency of microbial synthesis, NDF – neutral detergent fibre, ADF – acid detergent fibre

The values in a row with different superscript letters (a, b, c, d) differ at $P < 0.05$

acids such as α-linolenic acid (ALA) – $C_{18:3}$ and linoleic acid (LA) – $C_{18:2}$ (Table 1). The lipid composition of barley consists of triglycerides and the major fatty acids are oleic acid – $C_{18:1}$ and LA. The microbial oil was rich in $C_{18:1}$, evening primrose oil in LA, and borage oil in $C_{18:1}$ and $C_{18:2}$. All three oils used in this study contained γ-linolenic acid (GLA) (Table 1). The polyunsaturated fatty acids (PUFA) such as $C_{18:2}$ and $C_{18:3}$ are essential components of ruminant diets because they cannot be derived metabolically from oleic acid. Dietary lipids are extensively hydrolyzed in the rumen and PUFA are biohydrogenated by rumen microbes to more saturated products such as stearic acid – $C_{18:0}$ with the formation of intermediates like conjugated linoleic acid (*cis* 9, *trans* 11 $C_{18:2}$ – CLA) and vaccenic acid (*trans*11 $C_{18:1}$ – TVA) as the most important ones (Hartfoot and Hazlewood, 1988). In this study, the feeding oils significantly decreased the percentage proportion of SCFA (about 0.1–0.3%), MCFA (about 8%) and significantly increased (about 8%) the percentage proportion of LCFA (Table 3.). There were also changes in the profiles of some fatty acids among supplemental oils. Stearic acid ($C_{18:0}$) was the major FA in the effluent and the concentration of $C_{18:0}$ significantly decreased in oil supplemented diets (Table 3.). The lower proportion of $C_{18:0}$ in the ruminal effluent is an indication of lower biohydrogenation of unsaturated FA in the rumen. AbuGhazaleh et al. (2002) also found a lower proportion of $C_{18:0}$ in ruminal digesta when 2% menhaden oil or 1% menhaden oil + 1% extruded soybeans were added to TMR (total mixed ration) for cows. The accumulation of the *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 this experiment, the percentage proportion of *trans* $C_{18:1}$ isomers significantly increased (1.7–2 times) in all oil supplemented diets. This increase in *trans* $C_{18:1}$ isomers may be caused by the inhibition of reductase activity of ruminal microorganisms. AbuGhazaleh and Jenkins (2004) observed that the addition of DHA (docosahexaenoic acid), 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 proportion of *trans* $C_{18:1}$ isomers in ruminal

Table 3. Effects of microbial oil, evening primrose oil and borage oil on fatty acid production in the fermentation fluid in an artificial rumen (Rusitec)

FAME (%)	V_1 control (TMR)[a]	V_2 TMR+5% MO[b]	V_3 TMR+5% EPO[c]	V_4 TMR+5% BO[d]
SCFA	0.7 ± 0.1[b,d]	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
MCFA	30.3 ± 1.8[b,c,d]	22.2 ± 0.7	21.6 ± 0.8	23.4 ± 0.4
LCFA	68.9 ± 1.8[b,c,d]	77.3 ± 0.7	77.7 ± 0.7	76.1 ± 0.4
$C_{18:0}$	32.5 ± 1.4[b,c,d]	22.8 ± 0.2	15.0 ± 1.5	17.2 ± 1.7
$C_{18:1}$, *trans*	5.2 ± 0.7	10.7 ± 0.3[a,c,d]	8.8 ± 0.2	10.6 ± 1.0
$C_{18:1}$, *cis*	10.5 ± 0.3	28.1 ± 0.9[a,c,d]	14.3 ± 0.2	19.1 ± 0.9
TVA	1.4 ± 0.1[c,d]	3.2 ± 0.7	8.2 ± 0.7	9.4 ± 0.5
CLA	0.2 ± 0.3[b,c,d]	0.5 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
$C_{18:2}$, non-conj.	2.1 ±0.6[b]	6.0 ± 0.7	2.8 ± 0.2	3.9 ± 0.2
$C_{18:2}$, conj.	6.4 ± 0.6[b,c]	7.9 ± 0.2	14.6 ± 0.5	8.4 ± 1.1
$C_{18:3}$	1.1 ± 0.3	0.4 ± 0.0	0.7 ± 0.1	0.4 ± 0.0
$C_{20:5}$	0.1 ± 0.1[b,c,d]	1.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
UFA	36.9 ± 0.7	53.7 ± 0.2[a,c,d]	63.0 ± 0.8	59.1 ± 1.6
SFA	62.7 ± 0.5	46.0 ± 0.3[a,c,d]	37.1 ± 0.8	40.8 ± 1.5
SFA/UFA	1.7 ± 0.1	0.8 ± 0.0[a,c,d]	0.6 ± 0.0	0.7 ± 0.0

TMR – total mixed ration; MO – microbial oil; EPO – evening primrose oil; BO – borage oil; SCFA ($C_{4:0}$ – $C_{12:0}$); MCFA ($C_{14:0}$ – $C_{16:1}$); LCFA > $C_{18:0}$; SFA – saturated fatty acids; UFA – unsaturated fatty acids; CLA – *cis* 9, *trans* 11 $C_{18:2}$; TVA – *trans* 11 $C_{18:1}$

The values in a row with different superscript letters (a, b, c, d) differ at $P < 0.05$

digesta increased when cows were fed a mixed diet supplemented with fish oil (1%) and fat (2%) high in linoleic, linolenic and oleic acid (AbuGhazaleh et al., 2003). In our study, the concentration of *cis* $C_{18:1}$ isomers (*cis* 9, *cis* 11, *cis* 15) significantly increased in oil supplemented diets – 2.7, 1.4, and 1.8 times after microbial oil, evening primrose oil and borage oil supplementation. 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 biohydrogenation of other PUFA in the diet, resulting in the accumulation of *trans* $C_{18:1}$ isomers. *Trans* vaccenic acid – TVA (*trans* 11 $C_{18:1}$) was the major *trans* $C_{18:1}$ isomer representing 29.6, 92.6 and 88.4%, respectively, of total *trans* $C_{18:1}$ isomers in microbial oil, evening primrose oil and borage oil supplemented diets compared to 26% in TMR diet (Table 3). Borage oil, evening primrose oil and microbial oil supplementation of the diet resulted in a higher TVA concentration (9.37, 8.19 and 3.17%) in the rumen fluid (effluent) compared to the control (1.38). This increase in TVA concentration and in TVA to $C_{18:0}$ ratio from 0.04 (control) to 0.14 (microbial oil), 0.55 (evening primrose oil) and 0.55 (borage oil) is an indication of incomplete biohydrogenation of unsaturated FA with fat supplements. Previously, researchers (Chouinard et al., 1998; Dhiman et al., 2000; Donovan et al., 2000) used soybean oil, sunflower, linseed and fish oil to increase ruminal production of TVA. The concentration of the other main isomer – *cis* 9, *trans* 11 $C_{18:2}$ (CLA) significantly increased 2.3, 1.2, and 2.1 times after microbial oil, evening primrose oil and borage oil supplementation in the effluent in Rusitec. In cows fed a high concentrate diet with 5% linseed (LO), sunflower (SO) and fish oil (FO) Loor et al. (2004) found that SO feeding resulted in a higher CLA concentration (1.46%) compared with LO and FO. We can state that the extent of biohydrogenation of unsaturated FA in this experiment reflected: (a) the accumulation of *trans* fatty acids, especially TVA, in the effluent; (b) changes in the percentage of saturated and unsaturated FA in the effluent. Really, the concentration of unsaturated FA (UFA) significantly increased and the concentration of saturated FA (SFA) significantly decreased in all oil supplemented diets compared to the control. Thus, the ratio of SFA/UFA significantly decreased 1.98, 2.86, and 2.45 times with microbial oil, evening primrose oil and borage oil supplementation of the diet in Rusitec.

These conclusions can be drawn: (a) the supplementation of microbial oil, evening primrose oil and borage oil (5% wt/wt) to the total mixed ration did not affect the basal parameters of rumen fermentation (pH, methane and NH_3-N production, degradation of DM, OM, NDF, ADF) in Rusitec; (b) all used oils significantly decreased the percentage proportion of $C_{18:0}$, increased the production of *trans* $C_{18:1}$ isomers, mainly TVA, and increased the CLA production. These results indicate that biohydrogenation of unsaturated FA was incomplete and was depressed by the used oils.

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Received: 05–02–11
Accepted after corrections: 05–11–17

Corresponding Author

MVDr. Dusan Jalc, CSc., Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4–6, 040 01 Kosice, Slovak Republic
Tel. +421 556 336 251, fax +421 557 287 842, e-mail: jalcd@saske.sk