Effect of unsaturated C_{18} fatty acids (oleic, linoleic and α-linolenic acid) on ruminal fermentation and production of fatty acid isomers in an artificial rumen

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ABSTRACT: The objective of this study was to examine the effect of oleic (OA), linoleic (LA) and α-linolenic (ALA) acid used as supplements (3.5% wt/wt) to a diet containing 80% lucerne and 20% barley on rumen fermentation and lipid metabolism in an artificial rumen (Rusitec). The experiment lasted 12 days with 6 days of stabilization period. The fatty acid (FA) supplementation to a mixed diet did not affect any parameters of rumen fermentation (degradation of DM, NDF, ADF, total VFA production, production of acetate, propionate and butyrate). The methane production was decreased numerically (NS) by FA supplements (OA, LA, and ALA by about 8, 8.3 and 13.2%, respectively). The stoichiometric parameters of rumen fermentation such as N_M (nitrogen incorporated by microflora), OMF (organic matter fermented) and EMS (efficiency of microbial protein synthesis) were affected by unsaturated C_{18} FA to a different extent. EMS calculated from N_M and OMF was significantly ($P < 0.01$) increased by OA, ALA and decreased ($P < 0.01$) by LA. The lipid metabolism was also affected by C_{18} FA supplements. The concentration of total FA and proportion of LCFA (long chain fatty acids, > $C_{18:0}$) increased and proportion of MCFA (medium chain fatty acids, $C_{14:0}$ – $C_{17:0}$) decreased after OA, LA and ALA addition. The biohydrogenation (BH) of fatty acids was characterized by increased (NS) accumulation of stearic acid and *trans* isomers $C_{18:1}$ and lower SFA/UFA ratio in the effluent. The concentrations of two main BH intermediates, TVA (*trans* 11 $C_{18:1}$) and CLA (*cis* 9, *trans* 11 $C_{18:2}$), significantly ($P < 0.001$) increased (TVA – 1.06, 1.29 and 1.10; CLA – 0.32, 0.43 and 0.36 mg/g rumen fluid DM, respectively) after OA, LA and ALA supplementation compared to the control (TVA – 0.7; CLA – 0.23).

Keywords: fatty acids; rumen fermentation; lipid metabolism; CLA; TVA

The lipid composition of forages consists largely of glycolipids and phospholipids with major unsaturated fatty acids such as linoleic (*cis* 9, 12 $C_{18:2}$, LA) and α-linolenic (*cis* 9,12,15 $C_{18:3}$, ALA) acid. On the contrary, the lipids of seed oils used in concentrated feedstuffs are composed of triglycerides containing LA and oleic acid (*cis* 9 $C_{18:1}$, OA) as the predominant fatty acids (Bauman et al., 1999). When consumed by ruminants, dietary lipids undergo two important transformations in the rumen. The initial step is hydrolysis by microbial lipases and the second transformation is hydrogenation of

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unsaturated fatty acids (Hartfoot and Hazlewood, 1988; Jenkins, 1993). Biohydrogenation (BH) of C_{18} unsaturated fatty acids (OA, LA and ALA) in the rumen results in the production of primarily *trans* fatty acids and stearic acid ($C_{18:0}$) where several microorganisms are involved in this process (Loor et al., 2002; Kundrikova and Certik, 2005). Oleic acid is mostly hydrogenated to stearic acid, however, the conversion of oleic acid to *trans* 9 $C_{18:1}$ (elaidic acid) isomers by rumen bacteria has also been observed (Mosley et al., 2002). The biohydrogenation of LA is characterized by isomerization to *cis* 9, *trans* 11 $C_{18:2}$ isomer (conjugated linoleic acid, CLA), conversion of this isomer to *trans* 11 $C_{18:1}$ (*trans* vaccenic acid, TVA), and reduction to stearic acid ($C_{18:0}$). The biohydrogenation of ALA is characterized by its isomerization to *cis* 9, *trans* 11, *cis* 15 $C_{18:3}$ isomer and subsequent reduction via *cis, trans* isomers $C_{18:2}$,TVA and finally to stearic acid. Thus, the two key biohydrogenation intermediates, *trans* 11 $C_{18:1}$ (TVA) and *cis* 9, *trans* 11 $C_{18:2}$ (CLA), are formed from both LA and ALA. These intermediates are present in appreciable quantities in ruminant fat at the ratio of about 3:1 (Bauman et al., 2003). The effects of polyunsaturated fatty acids (PUFA) on rumen fermentation are well known (Tamminga and Doreau, 1991; Jenkins, 1993). Lipid supplementation of diets mostly reduces rumen degradation of fibre, and along with that of organic matter. Also, the inhibitory effect of PUFA in lipids is known for methanogenesis, when methanogens (Nagaraja et al., 1997), gram-positive cellulolytic bacteria and ciliates are reduced (McAllister et al., 1996) which provide hydrogen as a substrate for methanogens.

The aim of this work was to study the effect of unsaturated C_{18} fatty acids (oleic, linoleic and α-linolenic acid) as supplements to a pasture diet (fresh lucerne plus concentrate) on (a) rumen fermentation and (b) production of fatty acid isomers, mainly CLA and TVA in an artificial rumen (Rusitec).

MATERIAL AND METHODS

In vitro fermentation system

An 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 49.8 ± 1.8 kg) fed 960 g dry matter (DM) of lucerne and 240 g of crushed barley in two equal meals.

The chemical composition of fresh lucerne and barley was as follows: DM – 25.41 (89.69); nitrogen – 3.68 (2.18); ash – 12.09 (3.69); neutral detergent fibre (NDF) – 17.19 (26.32); acid detergent fibre (ADF) – 11.45 (6.79); hemicellulose – 5.73 (19.49); cellulose – 10.78 (5.41); lignin – 0.67 (7.72) as percentage of original DM. Fermentation inocula (solid and liquid) were collected through the rumen cannula immediately before 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 39.6 g (10.06 g DM) of fresh lucerne, 2.72 g (2.4 g DM) of crushed barley and 0.1 g of a mineral and vitamin mixture at daily intervals. Experimental treatments were as follows: 3.5% (wt/wt) addition of oleic acid* to vessel V_2, 3.5% supplementation of linoleic acid to vessel V_3 and 3.5% supplementation of α-linolenic acid to vessel V_4. The individual fatty acids were applied in drops, directly to the feed. The fermentation vessel V_1 served as control (without supplementation of fatty acids). A continual infusion of artificial saliva (pH 8.4) at the rate of 573–651 ml was maintained through each vessel during the experiment.

Free fatty acid preparation

One hundred g of sunflower or linseed oil was mixed with 1 l 2M ethanol solution of KOH and boiled under reflux for 1 hour. Then 1 l water was added and the mixture was extracted 3 times with 2 l hexane. The water/ethanol solution containing fatty acid potassium salt was blended with 1M HCl (pH 3) and released free fatty acids were extracted 3 times with 500 ml hexane. The hexane layer was filtered through anhydrous Na_2SO_4 and evaporated

*as a mixture of oleic acid and linoleic acid

under a reduced pressure. Unsaturated fatty acids were concentrated by urea treatment according to the modified method of Sajbidor et al. (1994). The following unsaturated fatty acids (UFA) were prepared by this method: linoleic acid (LA) of 94.42% purity (from sunflower oil) and α-linolenic acid (ALA) of 78.8% purity (from linseed oil). Besides prepared purified LA and ALA, commercially available oleic acid* (OA, Lachema Ltd., Brno, Czech Republic) of 51.8% purity was also used in this experiment (Table 1).

Measurements and chemical analyses

The experiment in Rusitec lasted 12 days. To ensure a steady state within the vessels a 6-day adaptation period followed by a 6-day collection period were used. On days 6–12 the following samples were collected. Produced gas was collected into special bags and gas volumes were measured with a gasmeter and methane concentrations were analysed in a gas chromatograph (Perkin-Elmer, Clarus 500) as reported by Czerkawski and Clapperton (1968). A liquid effluent was collected into flasks placed in ice bath and samples were taken for volatile fatty acid (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 the microdiffusion method (Conway, 1962). The fatty acid content in the effluent was determined in lyophilized samples. Lipids were extracted from 500 mg of freeze-dried effluent, lucerne and barley using chloroform and methanol (2:1, vol/vol) followed by 6 N HCL as described by Fellner et al. (1995). Fatty acids of total lipids as well as free UFA ($C_{18:1}$, $C_{18:2}$ and $C_{18:3}$, respectively) were analysed as their methyl esters (Christoperson and Glass, 1969) by gas chromatography according to Certik et al. (2006). Gas chromatograph (GC-6890N, Agilent Technologies, USA) was equipped with a capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and an FID detector (constant flow, hydrogen 35 ml/min, air 350 ml/min, 250°C). Analyses were carried out under a temperature gradient (130°C for 1 min; 130–170°C at program rate 6.5°C/min; 170–215°C at program rate 2.7°C/min; 220–240°C at program rate 2°C/min) with hydrogen as a carrier gas (flow 2.1 ml/min, velocity 49 cm/s, pressure 174 kPa) and a split ratio of 1/50 (inlets; heater 230°C, total hydrogen flow 114 ml/min, pressure 174 kPa). The fatty acid methyl ester peaks were identified by authentic standards of C_4–C_{24} fatty acid methyl ester mixture (Supelco, USA) and quantified by an internal standard of heptadecanoic acid ($C_{17:0}$, Supelco, USA). Dry matter, ash, nitrogen were analysed according to the methods of the Association of Official Analytical Chemists (AOAC, 1980). Neutral detergent fibre and acid detergent fibre in feed and residual feed samples were determined by the method of Goering and Van Soest (1970). Nitrogen (N) incorporated by the microflora (N_m) was estimated from the difference (mg/day) between total nitrogen (N_t, liquid overflow + bag feed residue) and NH_3–N plus urea-N ($N_m = N_t - (N_{NH3} + N_{UREA})$) and efficiency (mg/g) of microbial synthesis (EMS) = N_M/organic matter fermented (OMF) (Alves de Oliveira et al., 1997).

Statistical analysis

The means of values 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 Tukey-Kramer multiple comparison test. The tables show the group means and the standard error of the mean (± SEM). Probability values of $P < 0.05$ were considered as significant.

RESULTS

The addition of oleic acid* (OA), linoleic acid (LA) and α-linolenic acid (ALA) up to 3.5% in DM to a lucerne-barley (80:20%) diet had no effect on fermentation parameters such as pH of incubation fluid, degradation of dry and organic matter, degradation of NDF and ADF (Table 1). The methane production (mmol/day) was reduced numerically (not significantly – NS) about 8% by OA, 8.3% by LA and 13.2% by ALA supplements (Table 1). Total VFA production, production of acetate, propionate and butyrate (mmol/day), molar proportions (mol %) of acetate and butyrate, and the energetic efficiency of VFA were not affected by C_{18} unsaturated FA supplements. Only mol% propionate was

*as a mixture of oleic acid and linoleic acid

Table 1. Effects of oleic, linoleic and α-linolenic acid as supplements to a diet containing fresh lucerne and barley (80:20%) on the rumen fermentation pattern in Rusitec (n-6)

	Control[a]	Additives (3.5% wt/wt)			Pooled SEM
		OA[b]	LA[c]	ALA[d]	
Degradability (%)					
Dry matter	67.74	63.56	69.90	64.68	2.45
Organic matter	68.12	64.92	70.41	66.14	2.52
NDF	63.12	61.82	64.52	62.18	2.21
ADF	59.14	57.81	60.32	58.04	1.98
Fermentation parameters					
pH	6.93	6.87	6.73	6.89	0.04
VFA (mmol/day)	39.69	35.24	38.13	35.50	2.11
Acetate (mmol/day)	21.62	18.53	20.91	19.31	1.26
Propionate (mmol/day)	7.50	5.87	6.75	6.50	0.46
Butyrate (mmol/day)	6.46	6.54	6.50	5.96	0.36
Acetate (mol%)	54.40	52.58	54.70	54.47	0.86
Propionate (mol%)	18.82	16.66[a]	17.64	18.32[b]	0.38
Butyrate (mol%)	16.40	16.66	17.19	16.75	0.63
A/P ratio	2.89[c]	3.16[a]	3.10	2.96	0.04
Methane (mmol/day)	6.60	6.07	6.05	5.73	0.52
Ammonia (mmol/day)	18.2[d]	16.76	17.14	16.40	0.87
E (%)	75.42	74.96	75.05	75.27	1.90
N_M (mg/day)	85.33	86.73	73.44[a,b]	92.03[a,b,c]	2.42
OMF (g/day)	3.71[b,d]	3.38[c]	3.61[d]	3.36	0.08
EMS = N_M/OMF	23.0 [b,c,d,]	25.66 [c,d]	20.34 [d]	27.38	0.62

NDF = neutral detergent fibre; ADF = acid detergent fibre; VFA = volatile fatty acids; A/P ratio = acetate to propionate ratio; E = energetic efficiency of VFA; OMF = organic matter fermented; N_M = nitrogen incorporated by microflora; EMS = efficiency of microbial protein synthesis; ± SEM = standard error mean; values in a row with different superscript letters (a, b, c, d) differ at $P < 0.05$

decreased significantly by OA supplement (Table 1). The acetate to propionate ratio was increased significantly by OA and LA supplementation, but not by ALA supplementation. The other results showed that the supplementation of oleic and linoleic acid decreased the ammonia nitrogen concentration in the effluent slightly (NS) and that of linolenic acid decreased significantly. Based on this study, the stoichiometric parameters of rumen fermentation – nitrogen incorporated by microflora (N_M), organic matter fermented (OMF) and efficiency of microbial protein synthesis (EMS) were affected to a different extent by C_{18} fatty acid supplements. Thus, N_M was unchanged by OA, decreased ($P < 0.01$) by LA, but increased ($P < 0.01$) by ALA supplementation. Organic matter fermented (OMF) was decreased ($p<0.05$) by OA and ALA, and decreased slightly (NS) by LA supplement to the pasture diet in Rusitec. Finally, EMS was increased ($P < 0.01$) by OA and ALA supplements, and decreased ($P < 0.01$) by LA.

This study included an attempt to identify the biohydrogenation intermediates produced in the rumen when the supplements (3.5% wt/wt) of oleic* (OA), linoleic (LA) and α-linolenic acid (ALA) to a pasture diet (lucerne plus barley, 80:20%) were

*as a mixture of oleic acid and linoleic acid

Table 2. Fatty acid composition of feed ingredients and supplements

FAME (%)	Lucerne	Barley	OA	LA	ALA
$C_{14:0}$	0.40	0.28	–	–	–
$C_{16:0}$	2.71	17.97	0.28	0.23	0.07
$C_{18:0}$	0.35	1.66	–	–	–
$C_{18:1}$	0.41	17.27	51.84	4.74	1.80
$C_{18:2}$	1.70	54.51	38.90	94.42	17.27
$C_{18:3}$	95.0	5.0	7.09	0.15	78.78

FAME = fatty acid methyl esters; OA = oleic acid; LA = linoleic acid; ALA = α-linolenic acid

used in Rusitec. The lipid composition of individual FA showed that oleic acid contained 51.84% of OA and 38.9% of LA; linoleic acid contained 94.4% of LA and linolenic acid contained 78.8% of ALA and 17.3% of LA. The predominant FA in lucerne was ALA, but OA and LA were predominant FA in barley (Table 2). Overall, the supplementation of OA, LA and ALA to a pasture diet significantly increased the total FA concentration (mg/g of effluent DM) in the mixed rumen fluid-effluent (about 21.7%, 18.7% and 5.9%, respectively; Table 3). The supplementation of FA to the diet significantly decreased the percentage proportion of medium chain FA (MCFA, $C_{14:0}$–$C_{16:1}$, about 8.5–13.1%)

Table 3. Effects of oleic, linoleic and linolenic acid as supplements to a diet containing fresh lucerne and barley (80:20%) on the concentration (mg/g incubation fluid DM) of fatty acids and their isomers in Rusitec

Fatty acid	Control[a]	Supplements (5% wt/wt)			Pooled SEM
		OA[b]	LA[c]	ALA[d]	
$C_{14:0}$	0.16[b,c,d]	0.11	0.13	0.07	0.006
$C_{15:0}$	0.27[b,d]	0.20	0.26	0.19	0.008
$C_{16:0}$	1.62	1.35	1.45	1.15	0.04
$C_{16:1}$, c 9	–	–	0.13	0.15	0.01
$C_{17:0}$	4.89	5.03	4.94	4.80	0.14
$C_{18:0}$	2.43	2.91	2.93	2.72	0.19
$C_{18:1}$, t 11-TVA	0.71[b,c,d]	1.06	1.29	1.10	0.11
$C_{18:1}$, c 9	0.40[d]	0.33	0.27	0.23	0.02
$C_{18:1}$, c 11	–	0.17	0.21	0.30	0.01
$C_{18:2}$, c 9, c 12	0.45[b,c,d]	1.41	1.19	0.92	0.09
$C_{18:2}$, t 9, t 11	0.09[b,c,d]	0.32	0.28	0.20	0.02
$C_{18:2}$, c 9, t 11-CLA	0.23[b,c,d]	0.32	0.43	0.36	0.01
Total FA	11.02[b,c,d]	13.42	13.08	11.68	0.16
MCFA (%)	62.97	49.85	52.82	54.45	
LCFA (%)	37.02	50.15	47.17	45.54	
SFA (%)	85.03	71.53	75.30	77.74	
UFA (%)	14.97	28.46	24.70	22.25	
SFA/UFA	5.68	2.51	3.05	3.49	

OA = oleic acid; LA = linoleic acid; ALA = α-linolenic acid; TVA = *trans* vaccenic acid; MCFA = $C_{14:0}$–$C_{17:0}$; LCFA > $C_{18:0}$; SFA = saturated fatty acids; UFA = unsaturated fatty acids; c = *cis* isomers; t = *trans* isomers; ± SEM = standard error mean; values in a row with different superscript letters (b,c,d) differ at $P < 0.05$

and increased the percentage proportion of long chain fatty acids (LCFA, > $C_{18:0}$ about 8.5–13.1%). The concentration of unsaturated FA (UFA; mg/g of effluent DM) increased about 8–14% and the concentration of saturated FA (SFA) was reduced about 8–14% by FA supplementation compared to the control (Table 3). Thus, the ratio SFA/UFA decreased twice, 1.8, and 1.6 times after OA, LA and ALA supplementation compared to the control. The concentration of stearic acid increased slightly (NS) (approximately 1.2 times) in diets supplemented with the investigated fatty acids. Generally, three isomers of $C_{18:1}$ (*cis* 9; *cis* 11; *trans* 11) were determined and their concentrations were elevated by supplemented FA (Table 3). The main $C_{18:1}$ isomer – TVA (*trans*-vaccenic acid; *trans* 11 $C_{18:1}$) accounted for 67.9% (OA), 72.8% (LA) and 67.5% (ALA) of total *trans* $C_{18:1}$ isomers in the effluent compared to the control (64.5%). Moreover, the TVA concentration increased significantly ($P < 0.01$) when OA, LA and ALA were added to the diet (Table 3). Three LA isomers – conjugated (*trans* 9, *trans* 11; *cis* 9, *trans* 11) and non-conjugated (*cis* 9, *cis* 12) $C_{18:2}$ were also determined and their concentrations were markedly higher after FA supplementation (Table 3). The levels of the main CLA isomer (*cis* 9, *trans* 11 $C_{18:2}$) significantly ($P < 0.001$) increased (32.8, 50.0 and 40.2%) after OA, LA and ALA addition, respectively. CLA (*cis* 9, *trans* 11 $C_{18:2}$) was a predominant isomer and represented a 50–70% proportion of the total conjugated $C_{18:2}$ isomers.

DISCUSSION

Lipid supplementation of diets (dietary amounts higher than 5%) mostly reduces degradation of fibre and organic matter – OM (Sutton at al., 1983; Machmuller et al., 1998; Wachira et al., 2000). In our study, the rumen degradation of OM, DM and detergent fibre was similar in all fermentation vessels and it was not affected by C_{18} FA supplements. On the other hand, the supplementation of pasture diet with OA, LA and ALA (3.3% of DM) in a continuous fermenter showed that the treatments did not differ in DM digestibility, and the fibre digestibility was the same as or higher ($P < 0.05$) than in the control (Kolver et al., 2002). The methane production was reduced numerically (NS) (about 8–13%) by FA supplements. Similar results were presented in 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%), where an about 30% decrease (NS) in methane was observed (Jalc et al., 2002).

Czerkawski et al. (1966) found CH_4 reduction (27, 26 and 34%) caused by the infusion of 60 g/day of OA, LA and ALA, respectively, into the rumen of sheep fed dried hay. The ammonia concentration depends on ammonia use and release by a microbial population (Mansfield et al., 1995). Our results showed a decrease in NH_3-N concentration in the effluent after FA supplementation. This was probably caused by the suppression of ciliates and by their bacteria degradation activity after C_{18} FA supplements. The stoichiometric parameters of rumen fermentation – N_M, OMF and EMS were affected by C_{18} FA supplements differently. Several reports indicated a beneficial effect of PUFA (Sutton et al., 1983; Broudiscou et al., 1994) or a negative effect of PUFA (Czerkawski et al., 1975; Wachira et al., 2000) on the microbial protein synthesis. On the other hand, Kolver et al. (2002) found no effect of OA, LA and ALA supplementation (3.3% of DM) to pasture diet on the efficiency of microbial synthesis in a continuous fermenter.

The lipid metabolism in the rumen is characterized by lipolysis of dietary glycolipids, phospholipids and triglycerides leading to free fatty acids that are hydrogenated by microbes to saturated end products such as stearic acid ($C_{18:0}$) with the formation of intermediates like CLA and TVA (Hartfoot and Hazlewood, 1988). In our study, the supplementation of C_{18} FA to a pasture diet increased total FA concentration (mg/g of effluent DM) and the percentage proportion of LCFA and decreased the percentage proportion of MCFA in the incubation fluid in Rusitec. An increase of total FA in the rumen fluid is a typical response to lipid supplementation of ruminant diets (Beaulieu et al., 2002). The degree of biohydrogenation (BH) of individual FA was not estimated in this study; however many authors have reported different rates of BH after the addition of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ FA. For most diets, LA and ALA are hydrogenated to the extent of 70–95% and 85–100%, respectively (Doreau and Ferlay, 1994) and OA from 45% (Lee et al., 2003) for grass silage diet to 76% (Scollan et al., 2001) for a linseed oil supplemented diet. It is known that BH of PUFA is characterized by: (a) changes in UFA and SFA in the rumen; (b) accumulation of *trans* FA in the rumen; (c) higher concentration of stearic acid (AbuGhazaleh et al., 2002). All these three factors of BH were recorded (Table 3)

in our study. Moreover, our results showed that the TVA (*trans* 11 $C_{18:1}$) concentration significantly increased when OA, LA and ALA were added to the diet. The supplementation of LA caused a noticeable increase in TVA in the incubation fluid (Table 3). The accumulation of TVA was probably due to an excess of free fatty acids which inhibited the final hydrogenation of $C_{18:1}$ *trans* isomers to stearic acid (Gulati et al., 2000). Shingfield et al. (2003) also reported that the basal diet (silage and concentrate, 60:40%) supplemented with 500 g of rapeseed, soybean and linseed oil as the sources of OA, LA and ALA resulted in the formation of TVA as the most important isomer of total $C_{18:1}$ *trans* FA in cows. The concentration of the main CLA isomer – *cis* 9, *trans* 11 $C_{18:2}$ was significantly increased by OA, LA and ALA addition. Kolver et al. (2002) reported similar observations when LA and ALA increased CLA 15 and 5 times, respectively, after the continuous fermentation of high quality pasture diet enriched with LA or ALA. Oleic acid did not change the CLA concentration.

In conclusion, it can be stated that: (a) the supplementation of oleic, linoleic and α-linolenic acid up to 3.5% in DM to a lucerne and barley (80:20%) diet significantly increased the concentration of total FA, proportion of LCFA and reduced the proportion of MCFA; (b) FA biohydrogenation was characterized by the increased (NS) accumulation of stearic acid and *trans* isomers $C_{18:1}$ and lower SFA/UFA ratio in the effluent after FA addition; (c) the concentrations of two main BH intermediates – CLA (*cis* 9, *trans* 11 $C_{18:2}$) and TVA (*trans* 11 $C_{18:1}$) increased significantly ($P < 0.001$) after all FA supplements, mainly after LA, intermediately after ALA and the least after OA; (d) rumen fermentation was not affected by FA supplements, only the efficiency of microbial synthesis significantly increased after OA and ALA supplementation and decreased after LA supplementation.

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