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Rapeseed and fish oil mixtures supplied at low dose can modulate milk fatty acid composition without affecting rumen fermentation and productive parameters in dairy cows*

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The objective of this study was to supplement dairy cow diets with a mixture of rapeseed oil and fish oil at a low dose to modulate rumen and milk fatty acids without affecting other rumen fermentation and productive parameters. Our study was carried out on 14 lactating Polish Holstein-Friesian cows during their productive lives. One cow within each group was fitted with rumen cannulas. Animals were subjected to one of the treatments: 1) CON; total mixed ration (TMR) without oil supplementation, 2) FRM; consisting of TMR + 360 g/day/animal of rapeseed oil and fish oil in a 1:1 mixture. Milk production recorded throughout the experiment was not affected by oil mix supplementation; additionally, the whole tract digestibility did not differ when compared with the control group. No negative effects were also observed on the total rumen protozoan population, volatile fatty acids and

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methanogens; however, total bacterial counts were slightly affected (P>0.05) after 3h of feeding as an immediate effect of oil mix addition, which later disappeared at 6 h post-feeding. Feeding dairy cows with rich sources of long chain polyunsaturated fatty acids at a low dose (360 g/day/animal) resulted in favourable changes in milk fatty acid content without affecting milk fat concentration. Milk from dairy cows fed a diet with oil supplement had more (P>0.05) C18:2 c9t11 and C18:2 t10c12, by 30% and 38%, respectively, when compared to the control group. In addition, the level of supplement used in our study increased the n-3 fatty acids proportion significantly when compared to the control diet. Hence, we can conclude that using 360 g/day/animal (about 1.8% of dry matter intake) positively influenced the milk fatty acid content without any adverse effect on cows' productivity.

KEY WORDS: conjugated linoleic acid / fish oil / milk / rapeseed oil / unsaturated fatty acid

Evidently, a rising considerable public attention toward enhancing unsaturated fatty acid contents of animal products has become noticeable in the last few years. Previous studies have shown that supplementing ruminant diets with vegetable and fish oil mixtures increased polyunsaturated fatty acid contents in their products (milk or meat) [Potkanski *et al*. 2009, Strzałkowska *et al.* 2009, Jóźwik *et al*. 2010]. This growing interest is mainly connected with the potential benefits of n-3 long-chain fatty acids and conjugated linoleic acid (CLA) in particular to human health. Many studies highlighted that n-3 polyunsaturated fatty acids (PUFA) play a great role in decreasing the risk of coronary heart diseases [Poławska *et al*. 2011] as well as e.g. increasing cognitive development and mental performance in young children [Lopez-Huertas 2010]. Recent researches have also shown that C18:2 t10c12 isomer inhibits tumour growth in colon and gastric cancer cell lines, whereas the C18:2 c9t11 isomer may be efficacious in reducing the risk of breast cancer in premenopausal women [Koba and Yanagita 2013]. Additionally, studies published on the antidiabetic effect of conjugated linoleic acid isomers also indicated that either C18:2 c9t11 or C18:2 t10c12 increase insulin resistances in obese men with the metabolic syndrome. It has also been shown that C18:2 t10c12 are responsible for the body mass reduction and weight change [McCrorie *et al.* 2011]. The above-mentioned isomers can be provided to the human diet by products derived from ruminants (meat or milk). The CLA isomers present in milk fat arise from the rumen biohydrogenation process or from *de novo* synthesis in the mammary gland. However, increasing the dietary supply of polyunsaturated n-3 fatty acids (i.e. eicosapentaenoic EPA; docosahexaenoic DHA and linolenic LNA) has a limited effect due to the extensive transformation of these fatty acids in the rumen and low uptake by the mammary gland [Zymon *et al.* 2014], scientists are investigating the ways of positively modulated polyunsaturated fatty acid content in ruminants' products (milk and meat). However, this modulation may be accompanied by decreased rumen fermentation parameters, milk production and composition [Potkanski *et al.* 2009, Strzałkowska *et al*. 2010].

Most of the essential polyunsaturated fatty acids (PUFA) present in milk are not synthesized in ruminants' tissue, which facilitates modulation of PUFA concentrations using different dietary PUFA supplementation [Chilliard *et al.* 2007]. Several studies indicated that using fish oil alone or mixed with vegetable oils in ruminant diets leads to

an increase of n-3 contents in milk [Toral *et al.* 2010a]. However, fish oil may adversely affect milk yield by decreased dry matter intake. Potkanski *et al.* [2009] showed a significant increase of total rumen PUFA and a significant increase of milk DHA and conjugated linoleic acid supplementing the diet based on fresh alfalfa with a fish and rapeseed oil mixture. This increase could be a result of a combination of two effects; the inhibitory effect of fish oil that decrease C18 FA (fatty acid) biohydrogenation in the rumen and the accumulation of C18 FA in the rumen when dietary vegetable oils are provided [Toral *et al.* 2010a, 2010b, Shingfield *et al.* 2012].

We hypothesized that the effects of the fish and vegetable oil mixture on milk polyunsaturated fatty acid concentrations may be dependent on their unsaturated fatty acid contents and hence it is possible to modulate milk fatty acid composition without negatively affecting rumen fermentation parameters, milk production and composition. Therefore, the present study was conducted to determine the effects of a low dose of a mixture of fish oil (a source of essential long chain fatty acids) and rapeseed oil (a source of C18 fatty acids) as a dietary supplementation on basic rumen fermentation parameters and the milk fatty acid composition in lactating dairy cows.

Material and methods

Animals, experimental diets and management

Twelve non-cannulated (600±30 kg body weight) and two lactating Polish Holstein-Friesian cows fitted with rumen cannulas (600±25 kg body weight) at their 4th month of lactation were used. The experiment lasted 26 days; the first 23 days were for adapting the animals and its rumen microflora to the experimental diets, the following three days were for collecting samples. Diets were prepared daily according to the INRA standards to contain 1595 g protein truly digestible in the small intestine and 16.0 unit for milk production [INRA 1993]. The control diet (CON) consisted of a total mixed ration (TMR) based on alfalfa silage (10.4 kg of DM per day), meadow hay (1.6 kg of DM per day) and a concentrate (8.06 kg of DM per day; forage: concentrate ratio 60:40; DM basis), whereas the experimental diet (FRM) was based on the same components as in the control group, but additionally it was supplemented with 360 g of a 1:1 rapeseed oil and fish oil mixture. Diets were divided into two equal parts and fed daily at 8:00 a.m. and 4:00 p.m. During the whole experiment clean water was available *ad libitum*. Cows were milked daily at 4:30 a.m. and 4:30 p.m.

Sampling procedures and measurements

During the sampling period milk, rumen content and feces samples were collected. Cows were milked twice a day, milk yield at each milking was recorded and daily composite samples were prepared based upon a proportion of morning and evening yields. Milk samples for basic constituents were analyzed directly after milking (Milko-Scan 255 A/S N (Foss Electric, Hillerod, Denmark), whereas for fatty acid detection they were stored at -20°C prior to analyses. Rumen contents (500 ml) were

withdrawn through the cannula directly before and then 3 and 6 hours after morning feeding [Grummer 1991]. Immediately after withdrawal, rumen contents were strained through four layers of cheesecloth to obtain rumen fluid. The pH of rumen fluid was measured immediately after collection using a glass electrode (CP-104, Elmetron®, Zabrze, Poland). Ammonia concentration was determined using the Nessler method as described by Szumacher-Strabel *et al.* [2002]. For VFA, 3.6 ml of rumen fluid was stabilized with 0.4 ml of 46 mM mercury chloride ($HgCl_2$) solution and frozen at -20°C until analysis. The volatile fatty acid profile was determined using a gas chromatograph (GC Varian CP 3380, Sugarland, TX, USA). An Innowax 19091N-13 column (30 m, 0.25mm, 0.25 µm film thickness, Agilent HP) and a flame ionization detector were used. Helium was used as a carrier gas at a constant flow of 30 ml/min. The oven temperature was programmed as follows: initially 80°C for 1 min, then increasing at 15°C /min to 130°C, holding for 5 min and then increasing at 15°C /min to 240°C. A 2µl sample volume was injected into the column. The qualitative and quantitative identification of VFA peaks was made using the method based on external standards prepared by mixing individual VFAs purchased from Fluka (Sigma Aldrich, St. Louis, MO, USA) using the MS Work Station 5.0. The rumen fluid samples for fatty acid detection were stored at -20°C immediately after collection prior to analysis. Fatty acid composition in all samples was analyzed based on Cieslak *et al.* [2009] using a gas chromatograph (Varian Star CP 3800) fitted with a flame ionization detector and a 100-m fused silica capillary column (i.d. 0.25) coated with 0.2 µm of CP-Sil 88 (CHROMPACK, Varian).

The protozoa counts were determined according to Michalowski *et al.* [1986], using a drop of rumen fluid with a defined volume (100 µl) under a light microscope (Zeiss, type Primo Star no. 5, Jena, Germany). The bacteria were counted under a microscope (400×) in a Thoma chamber (0.02 mm depth, Blau Brand, Wertheim, Germany), according to Ericsson *et al.* [2000]. Rumen fluid for molecular analysis (FISH) was collected 3 h after morning feeding. The *Archaea* population, total methanogens, (S-D-Arch-0915-a-A-20) and two order-specific probes, S-O-Mmic-1200-a-A-21 (*Methanomicrobiales*) and S-F-Mbac-0310-a-A-22 (*Methanobacteriales*) were determined by the fluorescence *in situ* hybridization (FISH) technique, according to Stahl *et al.* [1995] with some modifications as described by Pers-Kamczyc *et al.* [2011].

Representative samples of the TMR and oil mix supplement were collected three times during the sample collection period. The samples were then stored at -20°C until analysis. Samples of the TMR were analyzed according to the AOAC [2007] for dry matter (method no. 934.01) and ash (method no. 942.05). Crude protein was determined using a Kjel-Foss Automatic 16210 analyzer (method no. 976.05) and crude fat using the Soxtec System HT analyzer (method no. 973.18). Neutral detergent fibre and acid detergent fibre were determined using the method of Van Soest *et al.* [1991]. Organic matter was calculated as the difference between dry matter and ash contents. Chemical composition of TMR is presented in Table 1, while the fatty acid composition of the total mixed ration and rapeseed fish oil mix are presented in Table 2.

Total feces were weighed and 100 g of feces of individual cows were collected for

analysis of DM, ash, ether extract, aNDF and OM using the same method as for feed.

Calculation

Methane production was calculated based on the theoretical fermentation balance for the measured molar proportion of VFA and OM fermented in the rumen according to the equation cited by Wolin [1960] as: 57.5 mol glucose = 65 mol acetate + 20 mol propionate + 15 mol butyrate + 60 mol CO_2 + 35 mol CH_4 + 25 mol H_2O.

Milk energy values were calculated using the equation cited by Tyrrell and Reid [1965], while desaturase indices were estimated according to Brogna *et al.* [2011]. The atherogenicity index was calculated according to Chilliard *et al.* [2003], whereas the thrombogenic index was calculated as described by Ulbricht and Southgate [1991].

Statistical analysis

The statistical analysis of basic rumen parameters was conducted based on the averages of the rumen parameters obtained from 3 sampling points for each cow, per day (before feeding, 3, and 6 h after feeding). Statistical analyses of total rumen fluid, Archaea and digestibility were performed on the pooled mean values of rumen fluid. The following unitrait linear model was applied:

$$y_{ijk} = \mu + O_i + P_j + (OP)_{ij} + e_{ijk},$$

where:

y_{ijk} – the *ijk*-th observation, μ is the overall mean;

O_i – the fixed effect of *i*-th oil, P_j is the fixed effect of *j*-th period;

$(OP)_{ij}$ – the fixed effect of an interaction of oil supplementation by period;

e_{ijk} – the residual connected with *ijk*-observation.

All pairwise multiple comparison procedures were applied using Tukey's test. All values are shown as means with the standard deviation and the pooled standard errors of the means.

The statistical analysis of milk fatty acid composition was conducted on the mean values of pooled samples from morning and evening milkings, whereas for milk production, yield and composition parameters it was performed on mean values of milk parameters obtained from 2 sampling points per day (morning and evening milking). The differences between the means were compared using Student's t-test. The results were considered significant when $P \leq 0.05$. All values are shown as means with the standard deviation and the pooled standard errors of the means.

These computations were performed using the SAS software package (version 9.3, 2014).

Results and discussion

Several studies in the last decades were conducted mainly to illustrate the role of oil supplementation on rumen biohydrogenation and other parameters, i.e. methane

mitigation. Most of the results positively highlighted the role of supplemented oils on different rumen parameters; unfortunately, some negative impacts were noted as a result of a high dose of these additions as decreasing fibre digestion and milk fat depression [Beauchemin *et al.* 2007, Hellwing *et al.* 2012, Storlien *et al.* 2012, Patra and Yu 2013]. We hypothesized that using a lower dose of rapeseed oil and fish oil in a 1:1 mixture added to the total mixed ration (TMR) will positively improve milk fatty acid content without affecting rumen fermentation parameters, milk production and milk composition. This improvement could be due to the abundance of polyunsaturated fatty acid (PUFA) in both oils. When evaluating the oil mixture in the present study the recommendation that the dietary fat content in dairy cattle diets should not exceed 6-7% of the dietary dry matter intake [NRC 2001] was taken into account.

A total mixed ration (TMR) was used as a basal diet (Tab. 1). The total fat content in the basal diet reached 718 g/day/cow, which represents about 3.5% of the average dry matter intake. The crude oils used in our study consisted of a 1:1 mixture of both rapeseed oil and fish oil rich in unsaturated fatty acids (Tab. 2), which were supplemented based on the recommended level by the National Research Council, USA.

Table 1. Chemical composition of the TMR used in the study

Item	Dry matter intake (kg)	Dry matter (g/kg)	Organic matter (g/kg DM)	Crude protein (g/kg DM)	Crude fat (g/kg DM)	aNDF (g/kg DM)
Alfalfa, silage	10.4	225	950	151	38	428
Meadow hay	1.60	904	923	101	14	67
Corn grain, ground	0.78	864	985	115	41	165
Dry brewer's grain	0.55	913	954	207	13	375
Protein concentrate	1.37	915	907	350	31	182
Wheat bran	0.53	879	946	166	40	450
Commercial concentrate	4.83	879	940	198	41	261

Table 2. Fatty acid profile in total mixed ration and rapeseed fish oils mix (g/100 g FA)

Item	TMR[1]	FRM[2]
C12:0	0.27±0.07	0.04±0.002
C14:0	0.59±0.10	2.29±0.07
C16:0	20.3±0.69	10.4±0.19
C18:0	2.79±0.57	2.13±0.02
C18:1c9	17.7±0.21	40.9±0.54
C18:2c9c12	42.3±1.41	13.1±0.26
C18:3c9c12c15	9.11±0.67	6.29±0.06
C20:1	0.05±0.04	2.59±0.03
C20:5	0.03±0.02	3.97±0.14
C20:4n-6	0.07±0.01	0.64±0.01
C22:5n-3	0.53±0.09	0.71±0.02
C22:6n-3	0.25±0.03	5.57±0.20

[1]TMR – total mixed ration used as the main substrate.
[2]FRM – treatment group with oils mixture (rapeseed oil and fish oil) supplementation.

Results of milk yields and milk compositions are shown in Table 3. As we hypothesized, the supplemented level of mixed rapeseed oil and fish oil (1:1) used in our investigation did not affect milk production comparing to the control diet; the same expected observations were noticed in the case of milk fat percentage and yield, which showed no differences (P>0.05) when adding the oil mix when compared to the control. The same tendency was found regarding results of the whole tract digestibility (dry matter, organic matter, fat and aNDF digestibility), which also showed no differences between supplementation and the control group (Tab. 4). These results were in line with those reported by AbuGhazaleh *et al.* [2002b], who cited no changes in milk production or composition when using 2% (on dry matter basis) of the addition consisting of fish oil and/or extruded soybean as a supplement to the dairy cattle diet. The results of whole tract digestibility suggest that the used level of addition (360 g/day/cow) did not affect dry matter, organic matter, fat or aNDF digestibility. That is consistent with some previous studies, suggesting that limited doses of dietary unprotected fat can be added without a drastic effect on digestibility, clarifying that the fermentation in the hindgut might have been compensated for reduced ruminal digestibility, which could result in no changes in total tract digestibility [Bateman and Jenkins 1998, Wachira *et al.* 2000].

Table 3. Effect of treatments on milk yield and compositions

Item	Control	FRM[1]	SEM	*P-value*
	mean±SD	mean±SD		
Milk yield (kg)	24.8±0.99	25.0±0.71	0.146	0.549
fat (%)	4.13±0.22	3.93±0.35	0.053	0.070
protein (%)	3.43±0.47	3.54±0.32	0.069	0.607
lactose (%)	4.75±0.20	4.82±0.24	0.038	0.728
total Solids (%)	12.1±0.48	12.2±0.53	0.086	0.645
SNF[2]	8.18±0.48	8.36±0.42	0.078	0.520
energy (Mcal/Kg)	0.73±0.03	0.71±0.04	0.006	0.204
energy yield (Mcal)	18.0±1.09	17.8±1.00	0.178	0.516
energy (Mj/kg)	3.04±0.14	2.98±0.15	0.025	0.204
energy yield (Mj)	75.4±4.56	74.4±4.18	0.744	0.516
Milk yields (g/day)				
fat	1022±64.4	973±84.9	13.73	0.077
crude protein	851±126	885±76.5	17.91	0.520
lactose	1177±65.4	1205±78.3	12.42	0.525

[1]FRM – treatment group with oil mixture (rapeseed oil and fish oil) supplementation.
[2]SNF – Solids non fat.

The effect of the dietary rapeseed oil and fish oil mix on rumen basic parameters is presented in Table 4. Using the rapeseed oil and fish oil mixture as a supplement in our study resulted in a non-significant effect on rumen pH values, ammonia or methane production. No significant effect was also observed on the rumen bacterial and protozoan total counts; however, a significant decrease in the total bacterial count was observed in samples collected at 3 h when compared to the control group. The same trend was also observed in the case of the volatile fatty acid proportion (acetate, propionate, isobutyrate, butyrate, isovalerate and valerate) as well as total VFA, which

seems to be not affected by the level of the oil mix added throughout our experiment. These results suggested that using a lower dose of the 1:1 rapeseed oil and fish oil mixture in dairy cattle diets produced no differences when compared to the control diet. Vafa *et al.* [2010] clarified that the diet supplemented with fish oil and canola oil had no significant effect on either rumen pH or ammonia. Other authors showed that the decrease in ammonia nitrogen, if present, is mostly related with the decrease in the total protozoan population [Oldick and Firkins 2000, Vafa *et al.* 2010], which in our

Table 4. Effects of oil mix supplementation on ruminal parameters and whole tract digestibility of cows

Item	Mean±SD	0h[2]	3h[2]	6h[2]	Total	SEM	*P-value*		
							group	time	group x time
pH	CON	7.10±0.08	6.48±0.18	6.47±0.09	6.68±0.32	0.063	0.349	<0.001	0.510
	FRM[1]	6.94±0.20	6.54±0.32	6.32±0.49	6.60±0.43				
Ammonia (mmol/l)	CON	13.6±2.28	26.2±4.29	16.6±1.84	18.1±5.93	0.986	0.81	<0.01	0.294
	FRM[1]	12.6±2.42	22.7±4.53	20.1±5.76	18.4±6.07				
Methane[3] (mol/mol glucose equivalent fermented)	CON	0.44±0.04	0.60±0.10	0.60±0.04	0.55±0.10	0.016	0.832	<0.001	0.979
	FRM[1]	0.44±0.06	0.59±0.03	0.60±0.07	0.55±0.09				
Total VFA (mmol/l)	CON	78.6±9.14	112±13.5	107±12.1	99.8±19.1	3.412	0.812	<0.001	0.856
	FRM[1]	82.0±19.1	98.9±9.97	110±22.7	101±22.3				
Acetate (%)	CON	65.5±3.22	63.6±6.32	65.9±4.77	63.8±5.76	1.122	0.547	0.811	0.810
	FRM[1]	68.7±15.2	62.1±7.43	61.3±8.83	62.4±7.68				
Propionate (%)	CON	16.7±3.60	18.6±2.38	17.5±2.02	17.2±2.22	0.418	0.597	0.649	0.699
	FRM[1]	19.3±3.44	17.9±2.79	17.4±2.49	17.7±2.81				
Isobutyrate (%)	CON	5.28±1.53	6.19± 1.95	4.84±0.27	5.65±2.58	0.393	0.830	0.902	0.784
	FRM[1]	6.17±2.10	5.25±2.09	5.59±2.56	5.47±2.18				
Butyrate (%)	CON	9.64±2.19	9.08±2.35	9.43±2.62	9.16±1.97	0.402	0.208	0.625	0.546
	FRM[1]	9.84±1.39	9.97±2.64	11.3±3.45	10.2±2.74				
Isovalerate (%)	CON	3.27±0.72	3.25±0.46	2.57±1.07	2.88±0.84	0.140	0.733	0.808	0.166
	FRM[1]	2.92±0.39	3.20±0.92	3.18±0.96	2.98±0.87				
Valerate (%)	CON	0.63±0.26	1.88±0.50	1.32±0.61	1.28±0.65	0.105	0.859	0.003	0.518
	FRM[1]	0.99±0.69	1.56±0.37	1.47±0.69	1.29±0.63				
Acetate/Propionate	CON	4.10±0.99	3.48±0.67	3.81±0.57	3.80±0.76	0.144	0.673	0.564	0.829
	FRM[1]	3.23±1.07	3.59±0.92	3.64±0.97	3.67±0.97				
Protozoa (x 10^4/ml)	CON	45.8±14.5	36.3±8.21	26.1±1.10	46.3±21.7	2.967	0.071	0.017	0.748
	FRM[1]	43.4±9.51	27.4±7.21	35.6±9.71	36.3±11.4				
Bacteria (x 10^8/ml)	CON	31.1±4.02	34.2a±5.34	31.6±2.94	30.6±6.19	1.541	0.137	0.352	0.337
	FRM[1]	40.1±13.8	23.3b±6.88	36.1±7.12	35.2±11.2				
Archea (x 10^8/ml)									
total Methanogens	CON	-	-	-	22.3±4.32	1.305	0.276	-	-
	FRM[1]	-	-	-	19.3±4.59				
methanomicrobiales	CON	-	-	-	7.47±1.63	0.503	0.053	-	-
	FRM[1]	-	-	-	5.62±1.14				
methanobacteriales	CON	-	-	-	11.2±2.30	1.065	0.993	-	-
	FRM[1]	-	-	-	11.2±4.95				
Whole tract digestibility									
DM digestibility	CON	-	-	-	64.4±6.23	4.895	0.093	-	-
	FRM[1]	-	-	-	59.7±0.85				
OM digestibility	CON	-	-	-	66.5±4.98	4.238	0.069	-	-
	FRM[1]	-	-	-	62.1±1.79				
fat digestibility	CON	-	-	-	76.5±8.79	7.713	0.391	-	-
	FRM[1]	-	-	-	72.5±6.62				
aNDF[4]	CON	-	-	-	47.0±7.96	7.246	0.120	-	-
	FRM[1]	-	-	-	40.5±5.13				

[1]FRM – treatment group with oil mixture (rapeseed oil and fish oil) supplementation.
[2]Samples were taken at 0, 3 and 6 h after morning feeding.
[3]Methane (mol/mol glucose equivalent fermented) estimated based on fermentation balance [Wolin 1960].
[4]aNDF – neutral detergent fibre.

case was not affected. This consequently reinforced our idea to modulate milk fatty acid composition by a low dose of supplements without any negative effect on either rumen or productive parameters. The study conducted by AbuGhazaleh and Ishlak [2013] on incrementally increased amounts (0.5%, 2% and 3.5% on the dry matter basis) of fish oil in continuous fermenters showed no significant effect on total VFA, butyrate, valerate and isovalerate or fermenters pH similarly to our results. However, a decrease of rumen total bacterial count at 3 h was observed, similarly like in our research, which could be explained by the immediate toxic effect of polyunsaturated fatty acids on rumen bacteria [Maia *et al.* 2010]. Overall, our data suggest that the level of the rapeseed oil and fish oil blend was too low to affect rumen basic fermentation parameters or milk production and/or basic composition, but high enough to modulate milk fatty acid contents.

In line with our hypothesis, the supplemented dose of the rapeseed oil and fish oil mix affected the milk fatty acid composition favourably (Tab. 5). Milk C18:1 t11 (TVA, trans vaccenic acid) content was affected by the dietary treatment. The concentration of TVA in milk fat was increased by 217% as a result of the rapeseed oil and fish oil mix supplementation when compared with the control diet. One of the main goals of our investigation was to determine the effect of supplementing a mixture of rapeseed oil and fish oil into the dairy cattle diet on the milk fat concentration of conjugated linoleic acid (CLA), especially C18:2 c9t11 and C18:2 t10c12 as the main CLA isomers. In our study, both C18:2 c9t11 and C18:2 t10c12 contents were significantly increased by 30% and 38%, respectively, when compared to the control. A significant increase in linolenic acid (C18:3 c9 c12 c15) was also noted in relation to the control. Additionally, unsaturated, monounsaturated and polyunsaturated fatty acids increased significantly, whereas saturated fatty acids decreased by the rapeseed oil and fish oil mix addition when compared to the control (Tab. 5). The level of docosahexaenoic acid (DHA) increased ($P<0.05$) six times with the oil mixture addition when compared to the control diet, which as a result of work on increasing ($P<0.05$) the proportion of milk omega-3 fatty acids in comparison to the control. The inclusion of the rapeseed oil and fish oil mixture in dairy cattle diets resulted in an increase in the polyunsaturated fatty acid contents, especially omega-3 fatty acids, which consequently increased ($P<0.05$) DI RA (VA+RA) and decreased ($P<0.05$) the value of the thrombogenic index (TI) as well as the atherogenic index (AI) (Tab. 6). Increasing the *trans* vaccenic acid (TVA) content in the rumen is one of the most challenging steps as TVA is an intermediate formed during the rumen biohydrogenation process, which works as a main precursor of the majority of conjugated linoleic acid produced endogenously in the mammary gland [Griinari *et al.* 2000]. Hence, TVA can be considered to be the most limiting factor in enhancing milk C18:2 c9t11 (CLA) [AbuGhazaleh and Ishlak 2013]. The TVA concentration in the rumen was doubled by the rapeseed oil and fish oil mixture when compared with the control diet, which probably suggests that TVA was incrementally produced by ruminal biohydrogenation [AbuGhazaleh *et al.* 2002b].

In ruminants, biohydrogenation of long chain unsaturated fatty acids (LCUFA) relates with the presence of conjugated linoleic acid isomers (CLA). CLA isomers

Table 5. Effect of supplementing oil mixes on the milk fatty acid profile (g/100 g FA)

Item	Control	FRM[1]	SEM	*P-value*
	mean±SD	mean±SD		
Saturated fatty acids				
C12:0	3.33±0.72	2.98±0.89	0.145	0.140
C14:0	11.8±1.52	11.1±1.61	0.286	0.296
C16:0	32.3±2.22	31.2±3.29	0.491	0.309
C18:0	9.63±3.25	8.18±1.73	0.479	0.121
Monounsaturated fatty acids				
C14:1 c9	0.21±0.05	0.23±0.08	0.011	0.412
C16:1 c9	2.86±1.17	2.66±0.44	0.158	0.530
C18:1 t10	1.28±0.93	1.38±0.56	0.699	0.699
C18:1 t11	1.06±0.62	3.36±1.07	0.234	<0.001
C18:1 c9	20.1±7.54	20.8±6.31	1.22	0.760
Polyunsaturated fatty acids				
C18:2 c9c12	2.18±0.62	2.11±0.56	0.097	0.753
C18:2 c9t11	0.20±0.08	0.26±0.08	0.014	0.033
C18:2 t10c12	0.13±0.06	0.18±0.08	0.010	0.006
C18:3 c9c12c15	0.81±0.28	1.94±0.73	0.140	<0.001
C20:1 c9	0.39±0.08	0.37±0.08	0.014	0.656
C20:4 n-6	0.15±0.04	0.15±0.07	0.010	0.896
C20:5 n-3	0.03±0.03	0.04±0.05	0.007	0.993
C22:6 n-3	0.04±0.04	0.24±0.11	0.013	<0.001
Long FA	44.4±3.96	47.4±4.81	0.807	0.036
medium FA	51.9±3.48	49.3±4.25	0.710	0.073
short FA	3.68±0.86	3.23±0.74	0.146	0.118
C12:0, C14:0, C16:0	47.4±3.26	45.3±4.45	0.697	0.145
SFA[2]	62.7±3.45	58.5±4.75	0.807	0.009
UFA[3]	37.3±3.45	41.5±4.75	0.807	0.009
MUFA[4]	33.1±3.12	36.2±4.20	0.692	0.031
PUFA[5]	4.13±0.70	5.32±0.76	0.16	<0.001
n-6	4.57±4.46	4.88±4.82	0.820	0.854
n-3	1.23±0.44	2.37±0.60	0.137	<0.001
n-6/n-3	3.53±1.52	2.07±1.72	0.319	0.02

[1]FRM – treatment group with oils mixture (rapeseed oil and fish oil) supplementation.

[2]SFA – total of SFAs (C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0).

[3]UFA – total of UFAs (C14:1, C15:1, C16:1, C17:1, C18:1 t5, C18:1 t6-8, C18:1 t9, C18:1 t10, C18:1 t11, C18:1 t12, C18:1 t15, C18:1 c9, C18:1 c11, C18:1 c12, C18:1 c13, C18:1 c14, C18:1 c15, C18:2 c9c12, C18:2 c9c15, C18:3 c9c12c15, C20:1 n-9, C20:3 n-3, C20:4 n-6, C22:1 n-9, C22:2, C22:5 n-3, C22:6 n-3, C24:1).

[4]MUFA – total of MUFA (C14:1, C15:1, C16:1, C17:1, C18:1 t5, C18:1 t6-8, C18:1 t9, C18:1 t10, C18:1 t11, C18:1 t12, C18:1 t15, C18:1 c9, C18:1c11, C18:1 c12, C18:1 c13, C18:1 c14, C18:1 c15, C20:1 n-9, C22:1 n-9, C24:1).

[5]PUFA – total of PUFAs (C18:2 c9c12, C18:2 c9c15, C18:3 c9c12c15, C20:3 n-6, C20:3 n-3, C20:4 n-6, C20:5 n-3, C22:2, C22:5 n-3, C22:6 n-3).

Table 6. Effect of supplementing oil mixes on desaturation, thrombogenic and atherogenicity indices of produced milk

Item	Control	FRM[1]	SEM	*P-value*
	mean±SD	mean±SD		
Desaturase indices (DI)	0.39±0.14	0.56±0.16	0.025	0.999
DI C14:1 cis 9/(C14:0+C14:1 cis 9)	0.02±0.01	0.02±0.01	0.001	0.251
DI C16:1 cis 9/(C16:0+C16:1 cis 9)	0.08±0.03	0.08±0.01	0.004	0.861
DI C18:1 cis 9/(C18:0+C18:1 cis 9)	0.63±0.22	0.70±0.16	0.034	0.347
DI RA/(VA+RA)	0.87±0.04	0.93±0.03	0.007	<0.001
Δ^9-desaturase C14:1/C14:0	0.02±0.01	0.02±0.01	0.001	0.251
Δ^9-desaturase C16:1/C16:0	0.09±0.04	0.09±0.02	0.005	0.797
Δ^9-desaturase C18:1/C18:0	2.25±1.10	2.24±1.16	0.198	0.483
Thrombogenic index	2.46±0.36	1.88±0.40	0.083	<0.001
Atherogenic index	2.24±0.33	1.94±0.41	0.060	0.03

[1]FRM – treatment group with oil mixture (rapeseed oil and fish oil) supplementation.

are naturally produced by rumen bacteria as intermediates in the biohydrogenation of dietary LCUFA, mainly linoleic acid C18:2, with C18:2 c9t11 being the predominant conjugated linoleic acid (CLA) isomer found in ruminant milk or meat [Bauman and Lock 2006]. CLA is formed also from the endogenous conversion of C18:1 t11 (trans vaccenic acid, TVA), another intermediate of rumen biohydrogenation of linoleic acid or linolenic acid (C18:3) by the Δ-9 desaturase enzyme in the mammary gland [Cieslak *et al.* 2010, Buccioni *et al.* 2012]. Oil supplements that were used in the present experiments are rich sources of polyunsaturated fatty acids such as linoleic (C18:2 c9c12) and linolenic (C18:3 c9c12c15) acids. Potkanski *et al.* [2009] cited that the presence of fish oil in the diet of dairy cattle as a rich source of long chain fatty acids is related to the changes in rumen microbial ecology, which interfere with CLA formation. Additionally, AbuGhazaleh and Holmes [2009] and AbuGhazaleh and Ishlak [2013] showed that the high content of docosahexaenoic acid (DHA) in the fish oil could be responsible for the accumulation of TVA in the rumen that will be consequently transformed endogenously into c9t11 CLA in the mammary gland. A significant increase in linolenic acid was observed when compared to the control could be explained by the high content of polyunsaturated fatty acids in the supplement, especially linolenic acid, which could be transferred directly from the diets.

Normally, DHA is found only in trace amounts (≤0.01%) in milk fat of cows fed conventional diets, whereas EPA and DPA are present at about 0.05 and 0.08%, respectively [Wright *et al.* 2007]. However, in the present study the DHA level was six times higher when compared to the control diet, which was consequently involved in significantly increasing both omega-3 fatty acids and the polyunsaturated fatty acid proportion when compared to the control. Omega-3 fatty acids have many health benefits, including the ability to decrease cardiovascular diseases, rheumatoid arthritis and decreasing the risk of cancer [Kremer *et al.* 1987, Lopez-Huertas 2010, McCrorie *et al.* 2011]. However, the proportion of an increase in DHA concentrations in milk fat was still low compared with the concentration of DHA in the dietary supplements, especially

in fish oil. This low transfer efficiency has been reported previously by Donovan *et al.* [2000], AbuGhazaleh *et al.* [2002a] and Whitlock *et al.* [2002], as well as by Zymon *et al.* [2014], and it has been attributed to both biohydrogenation of unsaturated fatty acids in the rumen and the association with plasma lipoproteins, which are not good substrates for mammary lipoprotein lipase [Mansbridge and Blake 1997]. Another possibility for this low transfer efficiency is that these fatty acids are preferentially partitioned towards other tissues in the body [Ashes *et al.* 1992]. In the present study, the high significant content of omega-3 fatty acids and CLA interprets the obtained high significant level of polyunsaturated fatty acids by 29% in relation to its level in the control group.

Ulbricht and Southgate [1991] clarified that C14:0, C16:0 and C18:0 fatty acids are thrombogenic. Huang *et al.* [2008] also showed the beneficial effect of dietary plant oil supplementation and stated that the addition of soybean oil (main source of C18:2 c9c12) decreased the thrombogenic index of dairy milk ($P<0.05$). In the case of our study the decrease ($P<0.05$) in both n6/n3 ratio, thrombogenic and atherogenic indexes could indicate the extent of health benefits we could obtain from the produced milk.

Supplementing lactating dairy cows' diets with 360 g/day/animal under experimental conditions resulted in favourable changes in milk fatty acid content without affecting the concentration of milk basic constituents or milk yield. Milk produced in our study was enriched in polyunsaturated fatty acids, especially CLA isomers C18:2 c9t11 and C18:2 t10c12, without causing milk fat depression. In addition, the decrease in both the n6/n3 ratio and the thrombogenic index value draws a conclusion that milk produced using lower levels of the rapeseed oil and fish oil mixture supplementation (about 1.8% of DM intake) could be treated as a food recommended in the prophylaxis of many diseases and a valuable constituent of various types of diets recommended by dieticians. However, our research and the analysis of literature data indicate that results are strictly dietary and supplement-dependent.

REFERENCES

1. ABUGHAZALEH A.A., SCHINGOETHE D.J., HIPPEN A.R., KALSCHEUR K.F., WHITLOCK L.A., 2002a – Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. ***Journal of Dairy Science*** *85*, 2266-2276.
2. ABUGHAZALEH A.A., SCHINGOETHE D.J., HIPPEN A.R., WHITLOCK L.A., 2002b – Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. ***Journal of Dairy Science*** 85, 624-631.
3. ABUGHAZALEH A.A., HOLMES L., 2009 – Altering dairy cows' milk fatty acid composition through grazing and oil supplementation. ***Milchwissenschaft*** 64(3), 241-244.
4. ABUGHAZALEH A.A., ISHLAK A., 2013 – Effects of incremental amounts of fish oil on trans fatty acids and Butyrivibrio bacteria in continuous culture fermenters. ***Journal of Animal Physiology and Animal Nutrition*** 98(2), 271-278.
5. A.O.A.C., 2007 – Association of Official Analytical Chemists. Official Methods of Analysis. 18th ed. Gaithersburg Maryland: USA, Horwitz W, Latimer W, editor.
6. ASHES J.R., SIEBERT B.D., GULATI S.K., CUTHBERTSON A.Z., SCOTT T.W., 1992 – Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. ***Lipids*** 27, 629-631.

7. BATEMAN H.G., JENKINS T.C., 1998 – Influence of soybean oil in high fiber diets fed to nonlactating cows on ruminal unsaturated fatty acids and nutrient digestibility. ***Journal of Dairy Science*** 81, 2451-2458.
8. BAUMAN D.E., LOCK A.L., 2006 – Conjugated linoleic acid: Biosynthesis and nutritional significance. In: Fox PF, McSweeney PLH, editors. Advanced Dairy Chemistry, Lipids. 3rd edition. New York: Springer; 2: pp. 293-136.
9. BEAUCHEMIN K.A., MCGINN S.M., PETIT H.V., 2007 – Methane abatement strategies for cattle: Lipid supplementation of diets. ***Canadian Journal of Animal Science*** 87, 431-440.
10. BROGNA D.M.R., NASRI S., BEN-SALEM H., MELE M., SERRA A., BELLA M., PRIOLO A., MAKKAR H.P.S., VASTA V., 2011 – Effect of dietary saponins from Quillaja saponaria L. on fatty acid composition and cholesterol content in muscle Longissimus dorsi of lambs. ***Animal*** 5, 1124-1130.
11. BUCCIONI A., DECANDIA M., MINIERI S., MOLLE G., CABIDDU A., 2012 – Review: Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. ***Animal Feed Science and Technology*** 174, 1-25.
12. CHILLIARD Y., FERLAY A., ROUEL J., LAMBERETT G., 2003 – A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. ***Journal of Dairy Science*** 86, 1751-1770.
13. CHILLIARD Y., GLASSER F., FERLAY A., 2007 – Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. ***European Journal of Lipid Science and Technology*** 109, 828-855.
14. CIESLAK A., KOWALCZYK J., CZAUDERNA M., POTKANSKI A., SZUMACHER-STRABEL M., 2010 – Enhancing unsaturated fatty acids in ewe's milk by feeding rapeseed or linseed oil. ***Czech Journal of Animal Science*** 55, 496-504.
15. CIESLAK A., MACHMÜLLER A., SZUMACHER-STRABEL M., SCHEEDER M.R.L., 2009 – A note on comparison of two extraction methods used to quantify the C18 fatty acids in feed and digesta of ruminants. ***Journal of Animal and Feed Sciences*** 18, 362-367.
16. DONOVAN C.D., SCHINGOETHE D.J., BAER R.J., RYALI J., HIPPEN A.R., FRANKLIN S.T., 2000 – Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. ***Journal of Dairy Science*** 83, 2620-2628.
17. ERICSSON M., HANSTORP D., HAGBERG P., ENGER J., NYSTROM T., 2000 – Sorting out bacterial viability with optical tweezers. ***Journal of Bacteriology*** 182, 5551-5555.
18. GRIINARI J.M., CORL B.A., LACY S.H., CHOUINARD P.Y., NURMELA K.V., BAUMAN D.E., 2000 – Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta (9)-desaturase. ***Journal of Nutrition*** 130, 2285-2291.
19. GRUMMER R.R., 1991 – Effect of feed on the composition of milk fat. ***Journal of Dairy Science*** 74, 3244-3257.
20. HELLWING A.L.F., SORENSEN M.T., WEISBJERG M.R., VESTERGAARD M., LUND P., 2012 – Can rapeseed lower methane emission from heifers? ***Acta Agriculturae Scandinavica, Section A - Animal Science*** 62(4), 259:262.
21. HUANG Y., SCHOONMAKER J.P., BRADFORD B.J., BEITZ D.C., 2008 – Response of milk fatty acid composition to dietary supplementation of soy oil, conjugated linoleic acid, or both. ***Journal of Dairy Science*** 91, 260-270.
22. IZ INRA, 1993 – Research Institute of Animal Production (Instytut Zootechniki). Feeding Standards for Cattle, Sheep and Goats. Omnitech Press, Warsaw.
23. JÓŹWIK A., STRZAŁKOWSKA N., BAGNICKA E., ŁAGODZIŃSKI Z., PYZEL B., CHYLIŃSKI W., CZAJKOWSKA A., GRZYBEK W., SŁONIEWSKA D., KRZYŻEWSKI J., HORBAŃCZUK J., 2010 – The effect of feeding linseed cake on milk yield and milk fatty acid profile in goats. ***Animal Science Papers and Reports*** 28 (3), 245-251.

24. KOBA K., YANAGITA T., 2013 – Health benefits of conjugated linoleic acid (CLA). ***Obesity Research & Clinical Practice***, http://dx.doi.org/10.1016/j.orcp.2013.10.001.
25. KREMER J.M., JUBIZ W., MICHALEK A., 1987 – Fish oil supplementation in active rheumatoid arthritis. A double-blinded, controlled, cross-over study. ***Annals of Internal Medicine*** 106(4), 497-503.
26. LOPEZ-HUERTAS E., 2010 – Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. ***Pharmacological Research*** 61, 200-207.
27. MAIA M.R., CHAUDHARY L.C., BESTWICK C.S., RICHARDSON A.J, MCKAIN N., LARSON T.R., GRAHAM I.A., WALLACE R.J., 2010 – Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. ***BMC Microbiology*** 18, 10-52.
28. MANSBRIDGE R.J., BLAKE J.S., 1997 – Nutritional factors affecting the fatty acids composition of bovine milk. ***British Journal of Nutrition*** 78(1), 37-47.
29. MCCRORIE T.A., KEAVENEY E.M., WALLACE J.M.W., BINNS N., LIVINGSTONE M.B.E., 2011 – Human health effects of conjugated linoleic acid from milk and supplements. ***Nutrition Research Reviews*** 24, 206-227.
30. MICHALOWSKI T., HARMEYER H., BREVES G., 1986 – The passage of protozoa from the reticulo-rumen through the omasum of sheep. ***British Journal of Nutrition*** 65, 625-634.
31. NATIONAL RESEARCH COUNCIL (NRC), 2001 – Nutrient Requirements of Dairy Cattle. 7th. rev. ed. Natl. Acad. Sci.: Washington, DC.
32. OLDICK B.S., FIRKINS J.L., 2000 – Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. ***Journal of Animal Science*** 78, 2412-2420.
33. PATRA A.K., YU Z., 2013 – Effects of coconut and fish oils on ruminal methanogenesis, fermentation, and abundance and diversity of microbial populations *in vitro*. ***Journal of Dairy Science*** 96, 1-11.
34. PERS-KAMCZYC E., ZMORA P., CIEŚLAK A., SZUMACHER-STRABEL M., 2011 – Development of nucleic acid based techniques and possibilities of their application to rumen microbial ecology research. ***Journal of Animal and Feed Sciences*** 20, 315-337.
35. POŁAWSKA E., MARCHEWKA J., COOPER R.S., SARTOWSKA K., POMIANOWSKI J., JÓŹWIK A., STRZAŁKOWSKA N., HORBAŃCZUK J., 2011 – The ostrich meat – an updated review. II. Nutritive value. ***Animal Science Papers and Reports*** 29 (2), 89-97.
36. POTKANSKI A., SZUMACHER-STRABEL M., CIESLAK A., 2009 – Effect of enrichment the summer feeding ration for milking cows with mixture of fish oil and rapeseed oil on selected rumen parameters and milk fatty acid profile. ***Animal Science Papers and Reports*** 27(2), 83-93.
37. SAS INSTITUTE INC., 2013. SAS® 9.4 In-Database Products: User's Guide, Third Edition. Cary, NC: SAS Institute Inc.
38. SHINGFIELD K.J., KAIRENIUS P., AROLA A., PAILLARD D., MUETZEL S., AHVENJARVI S., VANHATALO A., HUHTANEN P., TOIVONEN V., GRIINARI J.M., WALLACE R.J., 2012 – Dietary Fish Oil Supplements Modify Ruminal Biohydrogenation, Alter the Flow of Fatty Acids at the Omasum, and Induce Changes in the Ruminal Butyrivibrio Population in Lactating Cows. ***Journal of Nutrition*** 142(8), 1437-1448.
39. STAHL D.A., AMANN R.I., POULSEN L.K., RASKIN L., CAPMAN W.C., 1995 – Use of fluorescent probes for determinative microscopy of methanogenic Archaea. In: Sowers KR, Schreier HJ, editors. Archaea: A Laboratory Manual: Methanogens. New York: Cold Spring Harbor Laboratory Press; pp. 111-121.
40. STORLIEN T.M., HARSTAD O.M., NARVAEZ N., WANG Y., MCALLISTER T.A., 2012 – Effects of different oils and plant extracts on in vitro ruminal methane production. ***Acta Agriculturae Scandinavica, Section A - Animal Science*** 62(4), 300-304.

41. STRZAŁKOWSKA N., JÓŹWIK A., BAGNICKA E., KRZYŻEWSKI J., HORBAŃCZUK K., PYTEL B., HORBAŃCZUK J., 2009 – Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation. ***Animal Science Papers and Reports*** 27 (4), 311-320.
42. STRZAŁKOWSKA N, JÓŹWIK A., BAGNICKA E., KRZYŻEWSKI J., HORBAŃCZUK K., PYTEL B., SŁONIEWSKA D., HORBAŃCZUK J., 2010 - The concentration of free fatty acids in goat milk as related to the stage of lactation, age and somatic cell count. ***Animal Science Papers and Reports*** 28 (4), 389-396.
43. SZUMACHER-STRABEL M., POTKAŃSKI A., KOWALCZYK J., CIEŚLAK A., CZAUDERNA M., GUBAŁA A., JĘDROSZKOWIAK P., 2002 – The influence of supplement fat on rumen volatile fatty acid profile, ammonia and pH levels in sheep fed a standard diet. ***Journal of Animal and Feed Sciences*** 11, 577-587.
44. TORAL P.G., FRUTOS P., HERVAS G., GOMEZ-CORTES P., JUAREZ M., DE LA FUENTE M.A., 2010a – Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. ***Journal of Dairy Science*** 93, 1604-1615.
45. TORAL P.G., SHINGFIELD K.J., HERVÁS G., TOIVONEN V., FRUTOS P., 2010b – Effect of fish oil and sunflower oil on rumen fermentation characteristics and fatty acid composition of digesta in ewes fed a high concentrate diet. ***Journal of Dairy Science*** 93, 4804-4817.
46. TYRRELL H.F., REID J.T., 1965 – Prediction of the energy value of cow's milk. ***Journal of Dairy Science*** 48, 1215-1223.
47. ULBRICHT T.L.V., SOUTHGATE D.A.T., 1991 – Coronary heart disease: seven dietary factors. ***The Lancet*** 338, 985-992.
48. VAFA T.S., NASERIAN A.A., HERAVI MOUSSAVI A.R., VALIZADEH R., DANESH MESGARAN M., KHORASHADIZADEH M.A., 2010 – Effects of Different Levels of Fish Oil and Canola Oil on Productive Performance of Holstein Dairy Cows. ***Indian Journal of Animal Research*** 44 (2), 79-86.
49. VAN SOEST P.J., ROBERTSON J.B., LEWIS B.A., 1991 – Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. ***Journal of Dairy Science*** 74, 3583-3597.
50. WACHIRA A.M., SINCLAIR L.A., WILKINSON R.G., HALLETT K., ENSER M., WOOD J.D., 2000 – Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. ***Journal of Agricultural Science*** 135, 419-428.
51. WHITLOCK L., SCHINGOETHE D.J., HIPPEN A.R., KALSCHEUR K.F., BAER R.J., RAMASWAMY N., KASPERSON K.M., 2002 – Fish oil and extruded soybeans fed in combination increase CLA in milk of dairy cows more than when fed separately. ***Journal of Dairy Science*** 85, 234-243.
52. WOLIN M.J., 1960 – A theoretical rumen fermentation balance. ***Journal of Dairy Science*** 43, 1452-1459.
53. WRIGHT T.C., ODONGO N.E., SCOLLAN N.D., MCBRIDE B.W., 2007 – Nutritional manipulation of functional foods derived from herbivores for human nutritional benefits. In: Meng QX, Liu JX, Zhu WY. editors. Proceedings of the 7th International Symposium on the Nutrition of Herbivores. China Agricultural University Press: Beijing, China.
54. ZYMON M., STRZETELSKI J., SKRZYŃSKI G., 2014 – Aspects of appropriate feeding of cows for production of milk enriched in the fatty acids, EPA and DHA. A review. ***Journal of Animal and Feed Sciences*** 23, 109-116.