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Effect of enrichment the summer feeding ration for milking cows with mixture of fish oil and rapeseed oil on selected rumen parametres and milk fatty acid profile

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Two trials were conducted. In trial I three lactating Polish Holstein-Friesian (PF) cows fitted with rumen cannulae were used in a 3 x 3 Latin square design. In each feeding cycle animals were offered one of three following diets: (1) without supplementation (C diet – control), (2) C diet supplemented with fish and rapeseed oils blend 1:1 (FR diet), and (3) C diet supplemented with commercial protected fat (CPF diet). In trial II, ten lactating PF cows were offered C diet, and another ten – FR diet. In both trials fat supplements constituted up to 4% of the diet dry matter. FR diet strongly affected the fatty acid composition of rumen fluid and milk of cows. Content of oleic acid and other MUFAs increased, while that of total SFAs dropped in the rumen and, in both trials, in milk. The FR diet led to increase in the *c*9 *t*11 CLA (conjugated linoleic acid) isomer content of milk in both trials. In trial II the *t*10 *c*12 CLA concentration of milk increased in cows fed the FR diet. It is evident that during the summer feeding based on fresh green forage, good results regarding milk fatty acid content can still be improved by feeding rapeseed oil and fish oil.

KEY WORDS: cow / fatty acids / fish oil / milk / rapeseed oil / rumen

For several years scientists have been focusing their attention on possibilities of making fatty acid profile of milk more favourable from the point of view of consumers' health. It is highly recommended to increase the concentration of unsaturated and decrease the concentration of saturated fatty acids in milk and milk products. Some

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unsaturated fatty acids are believed to possess pro-salubrious properties and isomers of the conjugated linoleic acid (CLA) belong to this group. Foods with high level of biologically active compounds (e.g. CLA) are frequently referred to as functional foods. The definition 'functional food' describes products containing components that except traditional functions (e.g. energetic role of fat) play other, important roles [Duggan *et al*. 2002]. The role and function that individual CLA isomers can play is still investigated with novel analytical methods. Supplementation of diet of dairy cows with plant oils and/or fish oil can change mutual proportions between fatty acids of milk, reducing the content of saturated and increasing that of unsaturated fatty acids, including conjugated isomers of linoleic acid [AbuGhazaleh *et al*. 2002, 2003, Newbold *et al*. 2004]. These changes are now universally recommended by dieticians. In practice of dairy cow feeding, fat-rich additives cannot exceed certain critical level because of their possible negative effect on the activity of rumen microorganisms. During summer feeding of dairy cows, fresh green forage allows to reach relatively high level of unsaturated fatty acids of milk including CLA isomers [Lock and Garnsworthy 2003]. However, because of limited forage resources at the disposal for ruminant feeding, attempts are being made to find another methods of altering the fatty acid profile in dairy products. The effect in question can be achieved by the introduction of oils, e.g. rapeseed or fish oils into the diet of cows-in-milk [Mosley *et al*. 2002, AbuGhazaleh *et al*. 2003]. The objective of the present investigation was to determine the impact of addition of fish oil and rapeseed oil blend to summer ration for milking cows on the fatty acid composition of milk fat. The novelty of experiments conducted was to evaluate the combined effect of long-chain fatty acids present in fish oil and oleic acid that is present in rapeseed oil, and to confirm the possibilities of using commercially available crude oils in dairy cow summer feeding in Poland.

Material and methods

In trial I three lactating Polish Holstein-Friesian cows fitted with rumen cannulae and selected upon their daily milk yield (28±3 kg) and body live weight (603±5 kg) were used. The trial was conducted according to the 3 × 3 Latin square design. Each of three experimental cycles lasted 24 days, including 21-days preliminary period and three days of sampling. The control diet was composed of fresh alfalfa, maize silage and commercially available B-type concentrate supplemented with wheat bran, barley germs and maize meal. The diet was balanced and formulated according to INRA standards to contain 1735 PDIN, 1639 PDIE and 16.47 UFL [IZ-INRA 1993]. Programme INWAR version 1.0 and INRAtion version 2.63 of 1998 were used for calculation. In each feeding cycle individual cows were fed alternatively according to one of the following three variants: (1) without fat supplementation (control – C diet), (2) with the addition of fish oil (POLTRAN s.c. Kuslin Poland) with rapeseed oil (ADM SZAMOTUŁY sp. z. o.o. Wielkopolskie Zakłady Tłuszczowe Poland. Ltd.) blend 1 : 1 (FR diet) and (3) supplemented with commercial protected Bergafat fat T-

300, (Berg + Schmidt, Poland – CPF diet). Rapeseed oil was fed as a source of oleic acid (65.15% FAME), fish oil elevated the level of polyunsaturated long-chain fatty acids (45.70% FAME) whereas protected fat was rich in palmitic acid (70-80% of the sum of FA). A principle was adopted that the dietary fat content should not exceed 4% of the dietary dry matter, hence then the addition of fat was determined after fat content in C diet analysis. Because fat content was l.8% of the dietary dry matter, 2% of additional fat was supplemented. The animals received 560 g fat supplement to dry matter (280 g twice a day to concentreate feed). Refusals were recorded daily and feed samples were collected weekly. No concentrate refusals were stated. Feed samples were analysed according to AOAC [2005]. Animals were fed twice a day at 8.00 a.m. and 4 p.m. During the sampling period, rumen contents (200 ml) were withdrawn through the cannulae directly before and then 3 and 6 hours after morning feeding [Grummer *et al*. 1993]. Immediately after withdrawal, rumen contents were strained through four layers of cheesecloth to obtain rumen fluid in which the following determinations were made: pH – using a CP-1-4 type pH meter, ammonia nitrogen by the Nessler method [Szumacher-Strabel *et al.* 2002], and volatile fatty acids according to Tangerman and Nagengast [1996] using a gas chromatograph (VARIAN STAR CP 3800) with a Nukol™ – Bonded Free Fatty Acid Phase column (30 m × 0.25 mm, df 0.25 μm, SUPELCO). Cows were milked twice a day, milk yield in each milking was recorded and daily composite samples were prepared based upon a proportion of morning (AM) and evening (PM) yields. Composite samples were prepared in two parts for analyses. One part was refrigerated at 4°C and analysed for milk basic constituents (Milkoscan apparatus). Separate aliquots were stored at -20°C, freeze-dried, and analysed for fatty acids [Cieślak *et al*. 2009] using a gas chromatograph (Varian Star CP 3800) fitted with 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).

In trial II, twenty Polish Holstein-Friesian cows in the first lactation (week 6 post calving) were chosen based on daily milk yield (28±3 kg) and body live weight (603±5 kg). The trial lasted four weeks. Animals were divided into two groups. Control cows (n=10) were fed the control (C) diet identical as in trial I while the diet for experimental cows (n=10) was enriched with fish oil and rapeseed oil blend (1:1) added up to 4% of the diet dry matter (FR diet) During the last week of trial II individual milk yields were recorded at each milking. Daily composite samples of milk were prepared based on the proportion of morning (AM) to evening (PM) milking yields. Composite samples were prepared in two parts. One part was refrigerated at 4°C and analysed for milk basic constituents (Milkoscan apparatus). Separate aliquots were stored at -20°C, freeze-dried, and analysed for fatty acids as in trial I [Cieślak *et al*. 2009].

The data obtained were subjected to the analysis of variance using general linear model (GLM) procedure of SAS [1996]. Significance of differences between means was assessed at $P<0.05$. Differences among means were identified with Duncan test.

Results and discussion

Fat content of milk was found significantly lower in FR than in C and CPF cows in trial I (Tab. 1) and than in C cows in trial II (Tab. 5). Excess fat fed to cows can reduce fat [Baumgard *et al* 2000, Mackle *et al.* 2003] and protein [Peterson *et al* 2002] content of milk. Isomers of linoleic acid *c*9 *t*11 and *t*10 *c*12 as well as other fatty acids from the C18 family of *trans* configuration reduce the amount of fat in milk by limiting the synthesis of fatty acids with short and medium chain length [Grinarii *et al*. 1998]. Such effect was observed in both trials of this experiment, although a distinct increase in the amount of unsaturated fatty acids occurred.

Table 1. Basic composition of milk (%). Trial I

Compound	Control diet	Control diet supplemented with		Standard error of mean
		fish oil + rapeseed oil (blend 1:1)	commercial protected fat	
Fat	4.39[a]	3.84[b]	4.13[a]	0.01
Protein	3.58	3.50	3.67	0.02
Lactose	4.40[b]	4.85[a]	4.76[a]	0.02
Dry matter	12.37	12.19	12.56	0.09
Solids-non-fat	7.98[b]	8.35[a]	8.43[a]	0.01

[ab]Within rows means bearing different superscripts differ significantly at P≤0.05.

Table 2. Rumen metabolism indicators: pH, ammonia and volatile fatty acids (VFA) contents of rumen fluid. Trial I

Item	Control diet	Control diet supplemented with		Standard error of mean
		fish oil + rapeseed oil (blend 1:1)	commercial protected fat	
pH	7.19	7.13	7.11	0.11
N-NH_3 (mmol/L)	18.11	22.20	19.73	1.52
Total VFA (mmol/L)	94.15	89.12	95.16	2.30
Per cent				
acetic (A)	60.88	61.93	63.66	0.12
propionic (P)	24.20	26.09	24.64	0.09
butyric	15.70	15.48	16.34	0.01
isobutyric	0.90	1.05	0.98	0.18
valeric	1.50	1.70	1.97	0.01
isovaleric	2.19	1.92	3.58	0.35
A/P	2.51	2.37	2.58	0.03

Introduction of the oils mixture into diets (FR groups) required determination whether the additives affected negatively the processes taking place in the rumen. In trial I no negative changes were observed in rumen parametres (Tab. 2).

Fats applied in both trials strongly affected the fatty acid composition of rumen fluid (Tab. 3) and milk (Tab. 4 and 6) confirming the favourable fatty acid profile in cows fed fresh forage crop and oils blend supplement. Content of saturated fatty acids (SFAs) was found decreased, while that of unsaturated fatty acids (UFAs), in particular CLA isomers, increased, both in the rumen and in milk. Couvreur *et al*. [2006] found that CLA concentration increased linearly together with the increase of the grass forage proportion in the diet. Changes in the milk fat composition described in the present report appear particularly favourable due to the increase in the linoleic acid isomers *c*9 *t*11 and *t*10 *c*12. Both of the above isomers are believed to have a number of unique properties, among others – anti-carcinogenic [Aro *et al*. 2000, Ip *et al*. 2003] and anti-diabetic [Houseknecht *et al*. 1998]. Moreover, they may be valuable in curing obesity [Lee *et al.* 2007] and are believed to prevent diseases of the cardio-vascular system [Belury 2002, Parodi 2004]. Milk rich in these compounds will never be a medicine, but it can certainly be treated as a food recommended in the prophylaxis of the above-mentioned diseases and valuable constituent of various types of diets recommended by dieticians. Dhiman *et al*. [1999] and French *et al*. [2000] reported that feeding

Table 3. Fatty acid composition of rumen fluid (% of total fatty acids). Trial I

Acid (% FAME)	Control diet	Control diet suplemented with		Standard error of mean
		fish oil + rapeseed oil (blend 1:1)	commercial protected fat	
C18:0	28.03^{A}	21.19^{B}	25.45^{B}	1.65
C18:1	22.51^{B}	23.16^{A}	21.43^{B}	2.36
C18:1 *cis*	8.85^{B}	9.84^{A}	8.22^{B}	2.56
C18:1 *trans*	13.66	13.32	13.21	1.023
*c*9 *c*12 C18:2	5.65	5.37	5.76	2.45
*c*9 *c*12 *c*15 C18:3	3.12^{B}	5.09^{A}	3.70^{B}	1.24
C20:5 (EPA)	0.40	0.34	0.48	0.20
C22:6 (DHA)	0.45^{B}	0.64^{A}	0.18^{C}	0.01
SFA	61.70^{a}	53.44^{b}	64.95^{a}	2.26
MUFA	25.13^{A}	29.00^{A}	22.58^{B}	2.45
PUFA	11.17^{B}	17.56^{A}	12.47^{B}	0.74
MUFA + PUFA	36.30	46.56	35.05	4.23
*t*11 (VA)	10.81^{B}	17.01^{A}	9.38^{B}	2.31
*c*9 *t*11 (CLA)	0.49^{A}	0.64^{B}	0.44^{AB}	0.03
*t*10 *c*12 C18:2	0.23^{ab}	0.28^{a}	0.21^{a}	0.04

FAME – fatty acids methyl esters; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; SFA – saturated fatty acids*;* MUFA – monounsaturated fatty acids*;* PUFA – polyunsaturated fatty acids; VA – *trans* vaccenic acid*;* CLA – conjugated linoleic acid (rumenic acid).

^{aA}Within rows means bearing different superscripts differ significantly at: small letters – P<0.05; capitals – P<0,01.

Table 4. Fatty acid composition of milk (% of total fatty acids). Trial I

Acid (% FAME)	Control diet	Control diet supplemented with		Standard error of mean
		fish oil + rapeseed oil (blend 1:1)	commercial protected fat	
C18:0	19.86	19.88	18.00	1.56
C18:1	19.44^{B}	24.70^{A}	15.05^{C}	2.01
C18:1 *cis*	3.99	5.70	3.02	1.23
C18:1 *trans*	15.45^{B}	19.00^{A}	12.03^{C}	1.65
*c*9 *c*12 C18:2	8.65	9.15	7.35	2.34
*c*9 *c*12 *c*15 C18:3	2,68	2.99	2.40	0.50
C20:5 (EPA)	0.04	0.05	0.04	0.03
C22:6 (DHA)	0.10^{b}	0.18^{a}	0.12^{b}	0.02
SFA	60.19^{A}	56.89^{B}	63.98^{A}	2.35
MUFA	27.80^{B}	32.35^{A}	28.00^{B}	2.14
PUFA	7.94	10.76	8.02	0.70
MUFA + PUFA	35.74^{B}	43.11^{A}	36.02^{B}	2,54
*t*11 VA	9.09^{B}	15.19^{A}	8.12^{B}	0.56
*c*9 *t*11 CLA	2.65^{b}	4.76^{a}	1.97^{c}	0.02
*t*10 *c*12 C18:2	0.20^{b}	0.25^{a}	0.14^{c}	0.04

FAME – fatty acids methyl esters; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; VA – *trans* vaccenic acid; CLA – conjugated linoleic acid (rumenic acid).

^{aA}Within rows means bearing different superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Table 5. Basic composition of milk (%). Trial II

Compound	Control diet	Control diet supplemented with fish oil + rapeseed oil (blend 1:1)	Standard error of mean
Fat	4.12^{a}	3.62^{b}	0.04
Protein	3.13	3.12	0.08
Lactose	4.67	4.68	0.02
Dry matter	11.92	11.42	0.10
Solids-non-fat	7.80	7.80	0.01

^{ab}Within rows means bearing different superscripts differ significantly at $P\leq0.05$.

cows with fresh green forage led to increase of the CLA isomers content in their milk. This is attributed to the high content of UFAs in fresh plant material, mostly *c*9 *c*12 C18:2 and *c*9 *c*12 *c*15 C18:3, which are isomer precursors. The final results – milk

Table 6. Fatty acid composition of milk (% of total fatty acids). Trial II

Acid (% FAME)	Control diet	Control diet supplemented with fish oil + rapeseed oil (blend 1:1)	Standard error of mean
C18:0	17.86	19.88	2.34
C18:1	19.45^{B}	25.96^{A}	3.45
C18:1 *cis*	4.42	6.60	1.56
C18:1 *trans*	15.03^{B}	19.36^{A}	2.78
*c*9 *c*12 C18:2	9.95	10.13	2.78
*c*9 *c*12 *c*15 C18:3	2.73	2.44	1.01
C20:5 EPA[2]	0.04	0.05	0.02
C22:6 DHA[3]	0.12^{b}	0.22^{a}	0.01
SFA[4]	61.56^{A}	56.61^{B}	2.54
MUFA[5]	29.70^{B}	34.23^{A}	1.77
PUFA[6]	8.74	9.16	4.25
MUFA + PUFA	38.44^{B}	43.39^{A}	1.38
*t*11 VA[7]	9.12^{B}	19.15^{A}	1.02
*c*9 *t*11 CLA[8]	2.87^{b}	4.96^{a}	0.08
*t*10 *c*12	0.10^{B}	0.35^{A}	0.04

FAME – fatty acids methyl esters; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; VA – *trans* vaccenic acid; CLA – conjugated linoleic acid (rumenic acid).
^{aA}Within rows means bearing different superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

fatty acid composition – will also be affected by the composition of green forage, or diet which jointly decide about the course of the biohydrogenation processes in the rumen and the activity of desaturation process in the mammary gland [Boufaïed *et al*. 2003ab, Ribeiro *et al*. 2005]. The *trans* vaccenic acid (TV; *t*11 C18:1) formed in the rumen by biohydrogenation is the main substrate for endogenous synthesis of CLA in mammary gland [Griinari *et al*. 2000]. Fish oil and also long-chain fatty acids, e.g. EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) have a big potential to affect the biohydrogenation process. Responses to fish oil, and also to EPA and DHA separately, are related to the changes in rumen microbial ecology, especially to the presence and variation of *Butyrivibrio fibrisolvens* and *Fusocillus* [Wąsowska *et al*. 2006]. According to the latter author it may be possible to alter this population to decrease biohydrogenation without compromising other aspects of ruminal fermentation. In the present investigation the effect of experimental factors on microbial population was not tested and should be evaluated in further research using molecular methods.

In trial I, in group of cows fed the FR diet (fish oil and rapeseed oil blend), the oleic acid content of rumen fluid as well as of other MUFAs occurred higher

(P<0.01), while that of total SFAs – lower (P<0.05) than in remaining two groups. Similar relations were found in both trials in milk (P<0.01). In comparison with the control group, also the level of *t*11 C18:1 was markedly higher – by 57%, P<0.01 – in the rumen fluid and by 67% and 110% in milk after oils blend supplementation, in trial I and II, respectively. According to AbuGhazeleh and Holmes [2007] the inclusion of fish oil or rapeseed oil into cows' summer diet increased the UFAs content of milk, including *trans* vaccenic acid (VA) and CLA. In the authors' earlier *in vitro* investigations when alfalfa was used as a substrate [Potkański *et al*. 2003], application of both mentioned oils (especially their mixtures) increased VA and CLA contents in the rumen fluid several times. Moreover, what was also observed in this study, high proportions of oleic acid may also be incorporated into CLA in the rumen [Mosley *et al*. 2002]. In this study, the inclusion of the mixture of fish and rapeseed oils into the diet led to increase (P<0.05) the *c*9 *t*11 CLA isomer content in the rumen fluid by 30%, and in milk by 78% and 73%, in trial I and II, respectively. The applied oils failed to affect the level of *t*10 *c*12 C18:2 in the rumen, but increased its level in milk by 25% and 250% in trial I and II, respectively.

Highly significant increase in concentration of total PUFAs was found in rumen fluid in cows fed FR diet, whereas no such effect was identified in milk – concentration of total PUFAs was elevated, but the effect was not confirmed statistically.

Protected fat (CPF diet) significantly affected the composition of fatty acids either in rumen fluid or in milk (Tab. 3 and 4). Decrease in stearic acid, DHA and total MUFA in the rumen fluid occurred in CPF cows, whereas decrease in sum of C18:1 *trans* and, as a consequence, in oleic acid was observed in milk. Moreover, the milk of CPF cows showed the lowest level of *c*9 *t*11 and *t*10 *c*12 isomers compared to C and FR-supplemented group (trial I).

The question remains whether it is worth applying oil additives during summer feeding of cows. As an effect of our experiments it becomes evident that in summer, during the period of feeding fresh green forage, good results regarding milk fatty acid composition can still be improved by supplementing the diet with fish and rapeseed oils. However, such approach will only be justified if there is a niche on the market and a demand for special products of high functional usefulness increases. There are signs indicating that this perspective may be realized in near future.

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Wpływ dodatku mieszaniny oleju rzepakowego i rybiego do letniej dawki pokarmowej dla krów dojnych na poziom wybranych wskaźników płynu żwacza oraz koncentrację kwasów tłuszczowych w mleku

S t r e s z c z e n i e

Przeprowadzono dwa doświadczenia. Pierwsze (I) w układzie kwadratu łacińskiego na trzech krowach dojnych rasy polskiej holsztyńsko-fryzyjskiej z kaniulami założonymi do żwacza. Każde zwierzę żywiono kolejno trzema dietami – bez dodatku tłuszczu (dieta C, kontrolna), z dodatkiem mieszaniny oleju rzepakowego i rybiego 1:1 (dieta FR) oraz z dodatkiem tłuszczu chronionego (dieta CPF). W doświadczeniu drugim (II) nieprzetokowane krowy dojne tej samej rasy żywiono dietą C i FR (po 10 zwierząt). W obu doświadczeniach tłuszcz dodawano do poziomu 4% suchej masy dawki. Dodatek mieszaniny olejów do dawki kontrolnej zmienił koncentrację kwasów tłuszczowych w płynie żwacza w

doświadczeniu I, a także w mleku w doświadczeniu I i II. Stwierdzono wzrost stężenia kwasu oleinowego, jak również innych kwasów monoenowych, oraz obniżenie koncentracji sumy kwasów nasyconych. Podobnie, w obu doświadczeniach stwierdzono wzrost koncentracji izomeru *c*9 *t*11 CLA w mleku oraz w doświadczeniu I – w płynie żwacza. W doświadczeniu II stwierdzono wzrost koncentracji izomeru *t*10 *c*12 w mleku. Autorzy wnioskują, że w okresie żywienia letniego korzystne zmiany składu kwasowego tłuszczu mleka krów można spotęgować dodając do paszy mieszaninę oleju rzepakowego i rybiego.