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Influence of combined supplementation of cows' diet with linseed and fish oil on the thrombogenic and atherogenic indicators of milk fat

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The study was conducted on Polish Holstein Black-and-White cows. From a herd of about 300 animals maintained in a free-stall dairy shed, two groups of 10 were selected by the analogue method: primiparous (P) and second-lactation cows (SL), taking into consideration the stage of lactation (90±30 days) and daily milk yield (35.42±2,6 kg). During the first 7 days (that were considered the initial period – CTL) all the cows were fed the same TMR (*total mixed ration*) diet. From day 8 to 28 (considered the true experimental period – FOL) at the same time of the day each cow from both groups received individually 150 g fish oil and 250 g linseed oil. Milk samples were taken individually from each cow three times: on the last day of CTL and on day 14 and day 28 of FOL. As a result of supplementation with FOL, a decrease in the SFA content of milk was observed – for P from 62.610 to 56.70 and for SL from 63.50 to 53.67 g/100g of fat. The milk from P cows after 21 days of supplementation was characterized by a higher level of total CLA ($P\leq0.01$), FFA ($P\leq0.01$), SFA ($P\leq0.01$), SCFA ($P\leq0.01$) as well as atherogenic (AI), thrombogenic (TI) indices ($P\leq0.01$) and lover level of PUFA n-3 ($P\leq0.01$), PUFA n-6 ($P\leq0.01$), LCFA ($P\leq0.01$) and MCFA ($P \leq0.01$) compared to SL cows. In conclusion, introducing FOL to TMR diets for cows can improve the nutritive value of milk. Differences in the concentration of these milk components between the experimental groups indicate that it is significantly affected by the age of cows.

KEY WORDS: cows / fatty acid / fish oil / linseed / milk

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Key to abbreviations:

AI – atherogenic index;
TI – thrombogenic index;
CTL – control;
P – primiparous cows;
SL – second-lactation cows;
MUFA – monounsaturated fatty acid(s);
PUFA – polyunsaturated fatty acid(s);
UFA – unsaturated fatty acid(s);
SFA – saturated fatty acid(s);
SCFA – short chain fatty acid(s);
LCFA – long chain fatty acid(s);
MCFA – middle chain fatty acid(s);
total CLA – total conjugated linoleic acid; CLA cis-9, trans-11 + CLA trans-10, cis-12;
FFA – functional fatty acid(s).

It is possible to increase the content of bioactive components in milk through the use of traditional feeding systems or by supplementing the diet with animal and/or plant fats. The use of green forage is a natural way of enriching milk with unsaturated fatty acids [Nałęcz-Tarwacka 2006]. The addition of plant oils to the cows' diet [Flowers *et al*. 2008, Jóźwik *et al*. 2010] reduces the content of SFA and increases the content of LA ($C_{18:2}$ n-6) and LNA ($C_{18:3}$ n-3) of milk.

Supplementation of the cows' diet with fish oil contributes to a decrease in $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ and a simultaneous decrease of the content of TVA ($C_{18:1}$ *trans*-11), EPA ($C_{20:5}$ n-3) and DHA ($C_{22:6}$ n-3) in milk – Hristov *et al*. [2009]. Decreasing the SFA in cow's milk is beneficial to the health of consumers, as dietary fats and oils have been associated with an increased incidence of cardiovascular disorders. Alread *et al.* [2006], reported that dairy products with a lower AI and TI are less likely to lead to atherosclerosis or coronary thrombosis, thus being potentially healthier for humans.

Also, supplementation with sunflower oil, soybean meal [Nelson *et al*. 2009], linseed oil, fish meals and oils, linseed and combined supplementation with soybeans and fish oil [AbuGhazaleh *et al*. 2009] significantly increases the CLA and LNA levels in cows' milk.

The content of health-benefit components in the milk of ruminants depends on genetic factors [Strzałkowska *et al.* 2009a], the health status of the mammary gland [Strzałkowska *et al.* 2010], stage of lactation [Nałęcz-Tarwacka 2006, Strzałkowska *et al.* 2009b, Puppel *et al*. 2012] and type of diet offered [Strzałkowska *et al*. 2009c, Puppel 2011, Kuczyńska *et al*. 2012].

In the literature available no information was found on the combined supplementation of the cows' diet with fish oil and linseed. Moreover, in the studies reported so far, the lactation parity factor has also not been taken into consideration. The present study aimed at determining the effect of a combined fish oil and linseed diet supplement on the content of fatty acids in cows' milk and to determine its relation(s) with the age of cows.

Material and methods

Animals and sampling

The study was conducted on Polish Holstein Black-and-White cows. From a herd of about 300 animals, maintained in a free-stall dairy shed, two groups of 10 were selected by the analogue method: primiparous (P) and second-lactation cows (SL), taking into consideration the stage of lactation (90±30 days) and daily milk yield (35.42±2,6 kg). Cows from both groups were maintained together in a free-stall system in a separate part of the same barn. The cows had continuous access to water and the TMR diet was provided for *ad libitum* intake, dry matter intake (DMI) was monitored daily throughout the experiment. The treatments were: (1) the control, which was the TMR with no combined supplementation of fish oil and linseed; and (2) Experimental, which was the control with 150 g day^{-1} fish oil and 250 g day^{-1} linseed (referred to as FOL in the remainder of the paper).

During the first seven days (initial period – CTL) all the cows received the same TMR diet. Representative TMR samples (3) were pooled and stored at -20°C until analysed. Milk collected from all cows on 7th day (before supplementation) was treated as a control.

From day 8 to 28 (experimental – FOL) at the same time of the day each cow from both groups received individually 150 g fish oil and 250 g linseed oil. The supplemented diet was complemented with the mineral mix Bolifor Mg^{+} (80g/cow/day). Milk samples were taken individually from each cow two times during the experimental period: on day 14 (collecting 1) and 28 (collecting 2). Pooled milk samples collected individually from each cow over the whole day were taken during milking, proportionally to the yield. The samples were preserved using the mlekostat preparation. After milking, milk samples were immediately submitted to the Cattle Breeding Division (Milk Testing Laboratory) of the Warsaw University of Life Sciences) for analysis.

Analytical methods

The percentage of fat, protein and lactose of milk was determined using infrared spectroscopy, with a MilkoScan FT120 (FOSS ELECTRIC), Danmark.

The composition of fatty acids was determined in milk fat extracted at room temperature according to the Röse-Gottlieb procedure [AOAC 1990]. Fatty acid methylation was performed using the transestrification method by Kramer *et al.* [1997]. The identification with fatty acid standards and quantitative determination of individual fatty acids of crude fat was conducted in Hewlett Packard 6890 GC with HP Chem. Software and CP – select CB for FAME fused silica WCOT, 100m x 0.25 mm column, and detector FID. The helium carrier gas was maintained at a linear velocity of 25 cm/s. The separation was performed at a pre-programmed temperature. The remaining parameters were as follows: split sample injector (50:1); injector temperature 240°C; detector temperature 300°C [Puppel 2011].

Atherogenic (AI) and thrombogenic (TI) indices were calculated using the equations elaborated by Allred *et al*. [2006].

The sum of selected fatty acids was calculated using equations described by Puppel [2011] in which:

MUFA (*monounsaturated fatty acid*) = Σ $C_{10:1}$, $C_{12:1}$, $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{17:1}$, TVA, $C_{18:1}$, $C_{20:1}$;
PUFA (*polyunsaturated fatty acid*) = Σ LA, GLA, DGLA, CLA, LNA, AA, EPA, DPA, DHA;
UFA (*unsaturated fatty acid*) = Σ MUFA + PUFA;
SFA (*saturated fatty acid*) = Σ $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$;
SCFA (*short chain fatty acid) =* Σ $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$;
LCFA (*long chain fatty acid*) = Σ $C_{18:0}$, TVA, OA, LA, GLA, LNA, CLA, $C_{20:1}$, $C_{20:3n3}$, $C_{20:4n6}$, DGLA, EPA, DPA, DHA;
MCFA (*middle chain fatty acid*) = Σ $C_{12:0}$, $C_{12:1}$, $C_{14:0}$, $C_{14:1}$, $C_{15:0}$, $C_{15:1}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{17:1}$;
total CLA = CLA *cis*-9, *trans*-11 + CLA *trans-10, cis*-12;
FFA (*functional fatty acid*) = Σ BA, TVA, OA, LA, CLA, LNA, AA, EPA, DPA, DHA.

Statistical methods

The data obtained were analysed using a multi-factor analysis of variance (least squares) and the SPSS 12.0 packet software. Only interactions between factors showing a statistically significant effect ($P \leq 0.01$ or $P \leq 0.05$) were taken into consideration. The level of significance was determined after performing preliminary statistical analyses. The following model was used for milk composition and the FA analysis:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times B_j) + (A_i \times C_k) + (B_j \times C_k) + e_{ijkl}$$

where:

Y_{ijkl} – dependent variable;
μ – general mean;
A_i – treatment effect (*i*=1-2); 1 – control; 2 – FOL;
B_j – week effect (*j*=1-3); 1 – control, 2 – after day 14 of the supplementation period, 3 – after day 21 of the supplementation period;
C_k – lactation parity effect (*k* =1-2) 1 – primiparous, 2 – second lactation cows;
$(A_i \times B_j)$ – fixed interaction effect between treatment and week;
$(A_i \times C_k)$ – fixed interaction effect between treatment and age of cow;
$(B_j \times C_k)$ – fixed interaction effect between week and age of cow;
e_{ijkl} – random error.

Results and discussion

The chemical composition of the TMR ration is presented in Table 1. The fish oil was characterized by a high content of OA ($C_{18:1}$ *cis*-9), EPA and DHA, while the linseed was rich in OA, LA and LNA (Tab. 1).

Table 1. Ingredients, chemical composition and fatty acid content of diets

Composition	Treatment[1]		
	CTL	FOL	
Ingredient (% of DM)			
maize corn silage	52.12	50.81	
alfalfa silage	18.84	18.34	
corn silage	10.02	9.77	
soybean meal	5.67	5.53	
pasture ground chalk	0.57	0.56	
vitosa- vitamin mix [2]	0.86	0.84	
salt	0.29	0.28	
rapeseed meal	11.34	11.06	
magnesium oxide	0.29	0.28	
bolifor mg^{+}– mineral mix (kg/d) [3]	0.00	0.28	
linseed	0.00	1.40	
fish oil	0.00	0.84	
Chemical composition (% of DM)			
ash	4.50	4.39	
crude protein	8.69	8.66	
ADF	20.5	20.5	
NDF	34.1	34.8	
Ca	0.9	1.01	
P	0.4	0.6	
UFL per kg of DM	0.97	1.01	
Fatty acid (g/100 g of fat)		fish oil	linseed
$C_{14:0}$	1.80	5.02	0.06
$C_{14:1}$	0.84	0.22	0.01
$C_{16:0}$	27.5	18.00	5.98
$C_{16:1}$	0.18	6.02	0.09
$C_{18:0}$	18.91	0.31	4.84
OA ($C_{18:1}$ *cis*-9)	19.67	24.90	18.54
LA ($C_{18:2}$)	23.7	4.32	12.96
LNA ($C_{18:3}$)	2.87	2.90	55.56
EPA ($C_{20:5}$)	0.00	7.40	0.00
DPA ($C_{22:5}$)	0.00	0.80	0.00
DHA ($C_{22:6}$)	0.00	12.66	0.04

[1]Cows were fed a control diet, basal diet (CTL) and basal diet supplemented with FOL (150 g fish oil + 250 g linseed).
[2]Contained (on 1000g): Ca – 150 g, P – 100 g, Na – 50 g, Mg – 40 g, Zn – 9000mg, Mn – 7000 mg, Cu – 1000 mg, J – 100mg, Se – 50 mg, vitamin A – 1 200 000 j.m., vitamin D_3 – 120 000 j.m., vitamin E – 5 000 mg, vitamin K – 93 mg, vitamin B_1 – 80 mg, vitamin B_6 – 160 mg, vitamin B_2 – 110 mg, vitamin B_{12} – 1 000 mcg.
[3]Contained (on 1000 g): Mg – 24%, P – 13,5%, Ca – 1%.

Dry matter intake was similar for both diets, even when fish oil and linseed were added to the feeds. This corresponds with the results reported by Whitlock *et al*. [2006] and Bharathan *et al.* [2008].

The effect of the supplement used on milk yield and content of basic milk components is shown in Table 2. During the period during which the supplement

Table 2. Effect of a combined supplementation of fish oil and linseed on milk yield and the level FCM, ECM, MPY and MFY

Item (kg/d)	Treatment [1]						
	CLT		FOL				SEM [4]
			Collecting 1 [2]		Collecting 1 [3]		
	P	SL	P	SL	P	SL	
Milk yield	37.10^{A}	33.74^{B}	37.42	37.02	38.54^{A}	38.96^{B}	4.42
FCM [5]	37.10	34.23	36.27	35.24	34.38	35.14	4.21
ECM [6]	37.76^{A}	34.88^{A}	36.70	36.53	35.49	37.23	4.12
MPY [7]	1.328	1.224^{a}	1.282	1.362	1.332	1.480^{a}	0.17
MFY [8]	1.484^{a}	1.382^{b}	1.420	1.362	1.264^{a}	1.304^{b}	0.15

[1] Treatment – cows were fed a control diet, basal diet (CTL) and basal diet supplemented with FOL (150 g fish oil + 250 g linseed).
[2] Milk samples collected after 14th day of the supplementation period.
[3] Milk samples collected after 21th day of the supplementation period.
[4] SEM – standard error of the mean.
[5] FCM – fat corrected milk.
[6] ECM – energy corrected milk.
[7] MPY – milk protein yield.
[8] MFY – milk fat yield.
[aA...]Means in the same lines marked with the same letters differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

was offered an increase in milk yield was observed both in primiparous and SL cows (by 3.8% and 15.4%, respectively). The increase in milk yield could be related to an increased energy intake with higher FOL level. The combined supplementation of fish oil and linseed affected the FCM, ECM, MPY and MFY levels during the 21-day supplementation period compared to the control samples (Tab. 2). As a result of the supplementation used, the MFY level decreased from 1.484 to 1.264 in primiparous and from 1.382 to 1.304 in SL cows. In turn, the content of MPY increased in the milk of primiparous and SL cows (from 1.328 to 1.332 and from 1.224 to 1.480 kg/d**,** respectively). DePeters and Cant [1992**]** suggested that the reduction of milk protein is often caused by a dilution effect rather than a reduction in protein synthesis and is almost always associated with an increase in milk yield .

The effect of the supplement used on the content of fatty acid is presented in Table 3. The SFA content decreased significantly (P≤0.01) in the milk from both P and SL cows (from 62.610 to 56.70 and from 63.52 to 53.67 g/100g of fat, respectively). This is advantageous from the point of view of the health of consumers, especially as it did not include the level of BA ($C_{4:0}$), which has health-promoting properties. The BA level increased in the milk of cows from both groups; in the case of multiparous cows by as much as 27% (in primiparous cows only 5.2%). Also Kennelly [1996] reported a higher level of BA in the milk of cows fed linseed. As a result of the supplement offered, the level of $C_{16:0}$ in primiparous and SL cows decreased from 30.85 to 28.45 and from 31.27 to 28.98 g/100g fat, respectively (P≤0.05). The cow's diet plays a role in determining the degree of unsaturation of milk fat [Perfield *et al*. 2007]. Animals are capable of desaturating SFAs to unsaturated FAs due to the presence in their organisms

Table 3. Effect of FOL on the fatty acid composition (g/100 g of fat), activity of Δ-9 desaturase, AI and TI level of milk from primiparous (P) and second-lactation cows (SL)

Item (kg/d)	Treatment [1]						SEM [4]
	CLT		FOL				
			Collecting 1 [2]		Collecting 1 [3]		
	P	SL	P	SL	P	SL	
Fatty acid							
BA	1.81	1.43	1.39	1.69	1.91	1.82	0.23
$C_{16:0}$	30.85^{a}	31.06^{b}	30.12	29.31	28.45^{a}	28.95^{b}	1.01
$C_{18:0}$	9.58	11.32	9.29	10.72	8.14	9.92	1.23
total CLA	0.62^{AB}	0.60^{CD}	1.63^{Ae}	1.74^{CF}	2.22^{Be}	2.16^{DF}	0.05
PUFA n-3	0.40^{AB}	0.39^{CD}	0.48^{AE}	0.56^{CF}	0.60^{BE}	0.62^{DF}	0.02
PUFA n-6	2.67^{AB}	2.64^{CD}	2.88^{Ae}	2.65^{CF}	3.02^{Be}	3.81^{DF}	0.82
FFA	6.66^{AB}	6.12^{CD}	10.88^{AE}	13.01^{CF}	14.42^{BE}	13.96^{DF}	0.95
UFA	33.81^{AB}	35.86^{CD}	40.09^{A}	39.71^{CE}	41.19^{B}	43.79^{DE}	1.19
MUFA	30.12^{AB}	32.23^{CD}	35.10^{A}	34.76^{CE}	35.35^{B}	38.19^{DE}	1.07
PUFA	3.69^{AB}	3.63^{CD}	4.99^{A}	4.95^{CF}	5.84^{BE}	5.59^{DF}	0.24
SFA	62.61^{AB}	63.52^{CD}	58.41^{AE}	54.47^{CF}	56.70^{BE}	53.67^{DF}	1.66
SCFA	5.82^{AB}	4.98^{cD}	4.44^{AE}	5.20^{c}	5.36^{BE}	5.22^{D}	1.03
LCFA	42.16^{A}	43.46^{B}	43.79	43.71	46.88^{A}	46.99^{B}	1.12
MCFA	50.17^{A}	49.86^{B}	48.75	48.01	46.77^{A}	47.65^{B}	1.05
Index Δ-9 desaturase							
index Δ-9 (SCD)	0.31^{aB}	0.33^{cD}	0.34^{a}	0.36^{c}	0.38^{B}	0.40^{D}	0.01
$C_{14:1}$:$C_{14:0}$	0.09^{a}	0.08^{b}	0.10	0.11	0.11^{a}	0.13^{b}	0.01
$C_{16:1}$:$C_{16:0}$	0.04^{A}	0.04^{B}	0.06	0.06	0.07^{A}	0.09^{B}	0.01
$C_{18:1\ c9}$:$C_{18:0}$	2.24^{a}	2.06^{b}	2.25	2.32	2.54^{a}	2.80^{b}	0.23
CLA:TVA	0.30	0.36	0.50	0.42	0.81	0.41	0.02
Index							
AI	2.30^{aB}	2.15^{dE}	1.99^{ac}	1.94^{df}	1.76^{Bc}	1.63^{Ef}	0.12
TI	2.83^{aB}	2.80^{dE}	2.52^{ac}	2.40^{df}	2.19^{Bc}	2.12^{Ef}	0.14

[1] Treatment- CTL – control (TMR) with no combined supplementation of fish oil and linseed, FOL – control with 150 g /d fish oil and 250g/d linseed.
[2] Collecting sample of milk after 14th day of the supplementation period.
[3] Milk sample collected after 21th day of the supplementation period.
[4] SEM – standard error of the mean.
[aA...] Means in the same lines marked with the same letters differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

of the *stearoyl-CoA* desaturase enzyme (SCD). Almost all milk $C_{4:0}$–$C_{14:0}$ and about half of $C_{16:0}$ is synthesized *de novo* by the mammary epithelial cells. The addition of oils containing long-chain UFA reduced the milk fat per cent and inhibited the *de novo* synthesis of SFA in the mammary gland. Weakening of *de novo* FA synthesis is mediated through a reduction in the mammary of the synthetic activity of *acetyl-CoA carboxylase* and FA, and abundance of *acetyl-CoA carboxylase* mRNA.

Feeding cows with a combination of fish oil and linseed over a period of 21 days clearly affected the MUFA, PUFA and UFA content of milk (compared to the control animals) – Table 3. As a result of the applied supplementation, the UFA level in P and SL cows increased from 33.81 to 41.19 and from 35.86 to 43.79 g/100g of fat, respectively (P≤0.01) after 21 days of supplementing the diet with FOL. This supports the findings of Flowers *et al.* [2008], who reported an increase in UFA level when linseed was added to the TMR diet of lactating dairy cows. However, Allred *et al.* [2006] reported the lowest values of UFA (33.3 g/100g of fat) when fish oil was added and 41.7 g/100g fat in the case of a mixture of fish and soybean oil.

The milk of SL cows was characterized by a higher level of MUFA (an increase from 32.23 to 38.19 g/100 g fat, $P \leq 0.01$) and a lower level of PUFA – 5.59 g/100 g of fat ($P \leq 0.05$) after feeding the supplemented diet. In turn, after the introduction of the supplement, the milk of P showed an increase in the content of PUFA n-3 and PUFA n-6 by 66% ($P \leq 0.01$) and 88% ($P \leq 0.01$), respectively, compared to the initial level. In the milk of the SL cows it increased by 62% ($P \leq 0.01$) and 69% ($P \leq 0.01$), respectively. Allred *et al*. [2006] and Moate *et al*. [2008] recorded higher concentrations of PUFA n-3 fatty acids in the milk produced from diets containing fish products. Several CLA isomers and their precursors were also identified. The total CLA ($C_{18:2}$ *cis*-9, *trans*-11 + $C_{18:2}$ *trans*-10, *cis*-12) content increased in the milk from both primiparous and multiparous cows, by 3.56- and 3.5-fold, respectively ($P \leq 0.01$) after day 21 of the supplementation period (Tab. 3). Whitlock *et al.* [2002] reported that the level of *cis*-9, *trans*-11 CLA in milk decreased after 14 days when FO and extruded soybeans were fed, whereas AbuGhazaleh *et al.* [2004] observed that the concentration of *cis*-9, *trans*-11 CLA in milk increased until day 21 and then declined. This tendency has also been observed by the authors in other investigations and is concordant with the present results.

From the point of view of the physiology of human nutrition the most important are fatty acids called functional fatty acids (FFA). Pro-health properties are shown by the following: BA, OA, LA, CLA, LNA, TVA, GLA ($C_{18:3}$ n-6), EPA, DPA ($C_{22:5}$ n-3) and DHA As a result of the supplement offered, the level of FFA in the milk of primiparous and SL cows increased ($P \leq 0.01$) from 6.66 to 14.42 g/100g fat and from 6.12 to 13.96 g/100g fat, respectively, after 21 days of supplementation with FOL, what comprises an almost 2.16- and 2.28-fold increase compared to the initial level.

The SCFA concentration in milk fat of P cows receiving the supplement tested decreased ($P<0.01$) compared to the control group. This observation supports the findings of Cant *et al*. [1997], who reported a decrease in short-chain and an increase in long-chain FAs in the milk fat when the diets of dairy cows were supplemented with dietary fat. In turn, in the case of second-lactation cows, it was found that the SCFA level increased from 4.980 to 5.22 g/100 g fat, what was probably related to the increase of the BA content in their milk (Tab. 3). Also Baer *et al.* [2001] reported a decrease in SCFA content of milk when fish oil was added to the diet of lactating dairy cows. The research conducted by Chilliard *et al.* [2007] proves that the introduction of UFA into the diet of cows affects the *de novo* synthesis of SCFA and MCFA, elevates the concentration of 18-carbon acids and thus increases the level of UFA in cow's milk. It was found that the level of MCFA decreased, while the level of LCFA increased compared to the control samples in both primiparous and second-lactation cows. This has also been reported by other authors [Hristov *et al.* 2009, AbuGhazaleh *et al*. 2009].

The AI and TI indices proved to be highly significantly lower (P≤0.01) in both experimental groups (P and SL) when compared to the control animals. The AI level decreased in primiparous cows from 2.3 to 1.76 and from 2.17 to 1.74 in the second-lactation cows, while the TI from 2.83 to 2.19 in primiparous and from 2.82 to 2.23 in second- lactation cows (Tab. 3). The milk from the latter was characterized by a lower level of TI, what was probably related to a significantly higher MUFA level in milk fat which is considered to have strong antithrombogenic properties. The results reached by Allred *et al.* [2006] corroborate the AI and TI reduction when a supplement of fish oil is introduced. Milk is considered a risk factor for atherosclerosis and coronary heart disease because of its cholesterol and SFA content. Therefore, dairy products with a lower AI and TI are potentially healthier for humans.

Fatty acid unsaturation indices were calculated by expressing each product as a product-to-substrate ratio, using equations described by Allred *et al.* [2006]. The Δ-9 desaturase index has been used as an estimator of SCD (*stearoyl-CoA desaturase*) enzyme activity in dairy cows [Corl *et al*. 2001**].** There are four major products of Δ-9 desaturase activity in the mammary gland: $C_{14:1}$, $C_{16:1}$, $C_{18:1}$ *cis*-9, and $C_{18:2}$ *cis*-9, *trans*-11 CLA, which are produced from $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and TVA, respectively. In the present research an increase was observed in the Δ-9 desaturase index in milk of both primiparous and second-lactation cows (Tab. 3). Perfield *et al.* [2007] suggested that the *trans*-10, *cis*-12 and *trans*-9, *cis*-11 isomers of CLA lead to a decrease of the desaturase index, whereas the *cis*-9, *trans*-11 isomer does not. The $C_{14:0}$ is synthesized in the mammary gland and therefore $C_{14:1}$ can only be produced by desaturation through Δ-9 desaturase enzyme. The average Δ-9 desaturase activity for $C_{14:1}$:$C_{14:0}$ was 0.11 in primiparous and 0.13 in second-lactation cows. Other authors have reported Δ-9 desaturase activity for $C_{14:1}$:$C_{14:0}$ ranging from 0.048 to 0.085 [Allred *et al*. 2006]. Moreover, Bilby *et al.* [2006] found that changes in the rumen are the source of CLA in case of diet supplementation; the synthesis in the mammary gland is of less importance. SCD has been shown to be the rate-limiting enzyme in the conversion of palmitate to oleate acid in the bovine adipose tissue.

According to Towsend *et al.* [1997] the cow's age has no significant effect on the content of individual fatty acids in milk fat. However, the research described by Nałęcz-Tarwacka [2006] does not corroborate this thesis. Also Thomson and Van der Poel [2000] showed that milk from multiparous cows contained slightly more SFA and less MCFA, MUFA and PUFA compared to that from primiparous animals. Similar results were obtained in the present study.

Summarizing, the milk from primiparous cows fed over a period of 21 days a TMR diet supplemented with linseed and fish oil, was characterized by a higher level of total CLA, FFA, SFA, SCFA as well as AI and TI indices. Simultaneously, the milk from primiparous cows contained less PUFA n-3, PUFA n-6, LCFA and MCFA than that of second-lactation cows receiving the same diet. Differences in the concentration of these milk components between the experimental groups indicate that it is significantly affected by the age of cows.

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