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Influence of fish oil, palm oil and glycerol on milk fatty acid composition and metabolism in cows during early lactation

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Abstract

The aim of the study was to determine an influence of diet additives in a form of protected palm oil, protected fish oil or unprotected fish oil with glycerol in Polish Holstein-Friesian cows on milk fatty acid composition, metabolism, milk yield and milk composition. Milk production, milk fat, and milk protein did not differ statistically between the groups. A significant increase ($P<0.01$) in glucose level in blood was noted after application of unprotected fish oil with glycerol. The lowest concentration of β-hydroxybutyrate and non-esterified fatty acids, with the highest cholesterol and triacylglycerol concentration was observed in protected fish oil. An increase in the content of long-chain acids was observed in milk fat of cows receiving protected fish oil when compared to the group receiving palm oil and unprotected fish oil with glycerol. Concentration of trans-vaccenic acid (TVA) was higher ($P<0.01$) in protected fish oil and unprotected fish oil with glycerol when compared to palm oil group. These changes were corresponded by concentration of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) (1.71, 1.68 and 0.61 g/100 g of total fatty acids, respectively). Irrespectively of the form of fish oil administration, an increase in milk eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content was noted. The present experiment provides evidence that milk fatty acids can be manipulated via dietary fish oil or unprotected fish oil and glycerol application.

Keywords: dairy cow, fish oil, glycerol, fatty acids in milk, conjugated linoleic acid, metabolism

Introduction

Energy deficiency and/or decreased dry matter intake during the periparturient period result in increased lipolysis of deposited fat and release of non-esterified fatty acids (NEFA) to the blood (Garnsworthy *et al.* 2008). Decrease in dry matter intake in last week prepartum may amount even up to 30-35 % (Hayirli *et al.* 2002). However the cows at the beginning of lactation are potentially able to compensate low fodder intake when large energy concentration is found in a dose, and the fodder is characterised by well taste. Dry matter intake at the beginning of lactation increases gradually, however the rapid increase in milk production after calving causes negative energy balance occurrence (Garnsworthy *et al.* 2008, Grummer 2008). NEFA which are not a subject to β-oxidation in liver are re-esterified to triacylglycerols and transferred to bloodstream as very low density lipoproteins. An ability

for re-esterification increases in case of negative energy balance. The rate of very low density lipoproteins transport from cows liver is however low what results from small possibilities of synthesis of proteins (apoproteins B) controlling rate of very low density lipoproteins synthesis and release (Avramoglu & Adeli 2004). These mechanisms cause triacylglycerols accumulation in hepatocytes during negative energy balance. Moreover, the increase in NEFA concentration in blood causes the reduction in the fodder intake (Overton & Waldron 2004).

An addition of protected fat to diet of cows may limit negative energy balance and/or shorten its duration. Introduction of protected extruded soya and protected fat to feeding doses of cows induced an increase in yield and lactose content in milk, and lowered glucose concentration in blood (Hammon *et al.* 2008). However, the glucogenic diet vs. lipogenic one was effective in improving the calculated energy balance and decreasing plasma β-hydroxybutyrate (BHBA) and liver triacylglycerols (TAG) concentration, suggesting a reduced risk of metabolic disorders in multiparous dairy cows (van Knegsel *et al.* 2007). Except fat influence on metabolic transformations in cows, the rage of studies aimed at modification of fatty acids profile in milk fat have been conducted. These studies were focused on an increase in the content of polyunsaturated fatty acids (PUFA) in milk fat, mainly eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3) and *cis*-9, *trans*-11 CLA isomer of conjugated linoleic acid (Castañeda-Gutiérrez *et al.* 2007, Shingfield *et al.* 2006).

Fish oil or fish meal was introduced to the diets of ruminants to increase the content of long-chain n-3 PUFA, such as DHA and EPA, for health reasons, however the transfer coefficients of these acids from feed to milk are low (AbuGhazaleh *et al.* 2004). Fish oil was used in cows in an unprotected form or in a form of calcium soaps or fish oil on mineral carrier (Donovan *et al.* 2000, Chilliard *et al.* 2001, AbuGhazaleh *et al.* 2004, 2007, Castañeda-Gutiérrez *et al.* 2007, Heravi Moussavi *et al.* 2007, Kupczyński *et al.* 2011). The study concerned mainly the possibility of the modification of fatty acids content in milk fat. The stimulatory effect of fish oil on milk *cis*-9, *trans*-11 CLA has been attributed to its ability to inhibit the reduction of *trans* C18:1 to C18:0 in the rumen, which is largely responsible for an increase in the supply of vaccenic acid (TVA; *trans*-11 C18:1) (Loor *et al.* 2005). The *cis*-9, *trans*-11 CLA isomer is produced primarily from endogenous conversion of vaccenic acid, a biohydrogenation intermediate of linoleic and linolenic acid, via the enzyme Δ9-desaturase (Piperova *et al.* 2002). It was observed that fish oil caused an increase in a concentration of CLA and fatty acids of n-3 family (EPA and DHA) in milk fat (Castañeda-Gutiérrez *et al.* 2007). Thus, supplementation of fish oil was very effective in this type of diet modification (Donovan *et al.* 2000, AbuGhazaleh *et al.* 2003, 2007). Dietary supplements of Ca salts of fatty acids from soybean oil and linseed oil increased the CLA content of milk fat by three to five fold over the control diet (Chouinard *et al.* 2001). Milk *cis*-9, *trans*-11 CLA concentrations were increased 360 % when fish oil was fed at 2 % of diet dry matter (DM) (Donovan *et al.* 2000). However, there is a weak transfer of EPA and DHA from nutrition dose to milk and other tissues (AbuGhazaleh *et al.* 2004, Heravi Moussavi *et al.* 2007).

The aim of the study was to compare an influence of the application of protected saturated fatty acids (palm oil), protected polyunsaturated fatty acids (fish oil) or unprotected fish oil with glycerol in high-yielding Polish Holstein-Friesian cows on milk production, milk composition, metabolic transformations and on milk fat fatty acids profile, with particular emphasis on CLA and n-3 PUFA.

Material and methods

Experimental design and treatments

The field trial was performed on a farm of Polish Holstein-Friesian breed cows of Black-White variety (the 280 cows herd produced an average annual yield of 10 500 kg of milk, 3.7 % fat and 3.3 % protein). All procedures for this trial were approved by the 2nd Local Ethical Committee for Experiments on Animals in Wrocław, Poland.

Thirty six cows were selected for the experiment by an analogous method on the basis of lactation stage (multiparous in 2-3 lactation), daily milk yield in 1st week postpartum (29±5 kg) and body live weight (642±35 kg).

The cows were kept in the same environmental conditions and fed with TMR (total mixed ration) diet. Cows were fed twice a day (06:00 and 15:00). The chemical composition of experimental (basal) diets was determined by standard procedures (AOAC 2005). Nutritional value of the diet is presented in Table 1.

Table 1
Ingredient and nutrient composition of the experimental diet (basal diet)

Ingredient, % of DM	
Maize silage	39.79
Haylage silage	20.82
Lucerne silage	7.29
Soybean meal	7.52
Rapeseed meal	5.89
Corn grain silage	6.85
Brewer grain silage	6.26
Wheat straw	2.63
Mineral vitamin mix*	2.20
Sodium bicarbonate	0.75
Nutrient content	
NE_L, MJ/kg	20.18
Crude fat, % DM	3.14
Crude protein, % DM	17.9
Crude fibre, % DM	15.1
NDF, % DM	38.6
ADF, % DM	20.4
Ca, % DM	1.26
P, % DM	0.61

DM: dry matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, *Fatromix Bo (Fatro Polska, Kobierzyce, Poland), in 1 kg DM of supplement: Ca 100 g, P 100 g, Na 130 g, Mg 50 g, Zn 8 000 mg, Cu 1 500 mg, Mn 6 000 mg, J 120 mg, Se 30 mg, Co 20 mg, vitamin A 120 000 IU, D 1 600 IU, E 5 000 mg, B 210 mg, H 80 mg

Amount of individual feed components in the total mixed ration was adjusted on a weekly basis on changes in the dry matter DM content. Cows were fed ad libitum allowing 5 % refusals. Daily individual intake was recorded.

The experiment included clinically healthy cows starting from 1st week of lactation. Cows from particular groups (n=12) were given the following additives:

- group I (PrPO) – 300 g/d of protected palm oil (BergaFat; Berg+Schmidt, Poznañ, Poland), i.e. dose commonly used in dairy cows feeding,

- group II (PrFO) – 300 g/d of protected fish oil (Fatrofertil; Fatro Polska, Kobierzyce, Poland),
- group III (FO+G) – 300 ml/d of unprotected fish oil and 150 ml/d of glycerol per day.

The preparations were given to cows twice a day in equal doses, for a period of 8 weeks. The preparations used in groups PrPO and PrFO were in a solid form. Fish oil and glycerol were given in a liquid form. All preparation were added to TMR.

Protected fish oil used in group PrFO was salmon oil, not calcium soaps (the protection procedure is claimed by the manufacturer - Fatro Polska). Herring-sprat fish oil (group FO+G) was obtained during the process of fish meal production (herring, sprat, mackerel) in Agro-Fish Ltd. company (Krokowa, Poland). Concurrently, glycerol of FHU Over company (Łask, Poland) was used in group FO+G feeding (99.7 % of 1, 2, 3 – propantriol).

Fatty acid composition of fat supplements is presented in Table 2. Following extraction (Soxhlet methods), fatty acids in the samples were methylated and methyl esters were analysed using gas chromatograph (Agilent 5973, Agilent Tech. Inc., St. Clara, CA, USA) equipped with mass detector (GC/MS), capillary column (Agilent DB 224 MS, Agilent Tech. Inc., St. Clara, CA, USA) of the following parameters: 60 m × 250 µm × 0.25 µm. The injector temperature was 240 °C. The flow rate of gaseous carrier (helium) was 2.0 mL/min and the split flow ratio was 1:100 for the entire period of investigation. The peaks were identified and quantified by reference to fatty acids standards (Supelco, Bellefonte, PA, USA).

Table 2
Fatty acid composition of fat supplementations

Fatty acid	Protected palm oil % of total fatty acids	Protected fish oil % of total fatty acids	Unprotected fish oil % of total fatty acids
C12:0	0.25	nd	nd
C14:0	1.29	4.46	5.97
C14:1	nd	0.30	0.43
C15:0	nd	0.45	0.62
C16:0	56.56	20.63	15.69
C16:1	nd	5.01	3.52
C16:2	nd	0.83	nd
C17:0	1.22	nd	nd
C18:0	39.87	2.86	2.85
C18:1	0.20	30.21	22.86
C18:2	nd	14.17	7.28
C18:3	nd	3.34	3.84
C20:0	0.49	nd	0.19
C20:1	nd	6.16	2.94
C20:4	nd	nd	0.92
C20:5	nd	3.93	8.19
C22:0	0.06	nd	0.90
C22:1	nd	0.93	2.94
C22:5	nd	0.72	1.62
C22:6	nd	6.00	13.83

nd: not detectable or below 0.01 g/100 g of fatty acids

Milk production, composition and fatty acids analysis

The cows were milked in their stalls twice a day at 05:00 and 15:00. Milk yield was recorded daily in experimental period (DMI). Analyses of milk chemical composition: milk fat, protein and

lactose levels were performed on Bentley 150 - Infrared Milk Analyzer (Bentley Instruments, Inc., Chaska, MI, USA). Somatic cells count (SCC) was marked with use of Somacount 150 (Bentley Instruments, Inc., Chaska, MI, USA).

For determination of the milk fatty acids composition, milk samples were collected from ten animals of each group before the beginning of the experiment, and next in one-week intervals (8 samplings in total). Subsequently, milk samples were centrifuged at 3 000 g at 4 °C for 15 min. to obtain the fat for fatty acids analysis. Milk fat samples were stored at –20 °C until analyses. Fatty acids were extracted from milk fat using the method adapted from Deeth *et al.* (1983). Acetyl chloride was used as methylating reagent. The separation was performed using a gas chromatograph (Agilent Technologies 5973) equipped with mass detector (GC/MS), capillary column (Agilent DB-224 MS) parameters 60 m × 250 µm × 0.25 µm. The injector temperature was raised from 70 °C to 240 °C at a rate of 4 °C/min. The flow rate of gas carrier (helium) was 2.0 mL/min and the split flow ratio was 1:100 for the entire period of investigation. The peaks were identified and quantified by reference to fatty acids standards (Supelco).

Blood metabolite analysis and body condition score

Blood from cows was collected at the day of the beginning of the experiment (1 week postpartum) and after 4 and 12 weeks of an application of preparations. The blood was collected in morning hours before feeding by the external cervical vein puncture. The blood samples were centrifuged for 15 min at 3000 g at room temperature, and the serum samples were frozen (–20 °C) until the use. The laboratory analyses were done using Pentra 400 biochemical analyser (Horiba ABX Diagnosis, Montpellier Cedex, France). The following parameters were estimated in the blood serum:

- β-hydroxybutyrate acid (BHBA), nonesterified fatty acids (NEFA) by enzymatic method, Ranbut (Randox, Crumlin, UK)
- glucose by oxidase method with use of Horiba ABX reagents;
- triacylglycerols (TAG), total cholesterol by enzymatic methods, Horiba ABX reagents;
- aspartate aminotransferase (AST) and γ- glutamylotransferase (GGT) enzymes activity by kinetic method (Horiba ABX reagents);
- total bilirubin concentration by colourimetric method, Horiba ABX;
- total protein and albumin by colometric method, Horiba ABX.

Body condition was determined weekly throughout the study as the average of scores given by one individuals. Cows were body scored on a 5-point system (with an interval of 0.25).

Statistical analyses

The data obtained were subjected to the analysis of variance using general linear model (GLM) procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The statistical model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \quad (1)$$

where Y_{ij} is the dependent variable, μ is the overall mean, α_i is the effect of treatment (i=1, 2, 3), β_j is the series of analyses after experimental factor introducing, $\alpha\beta_{ij}$ is the effect of the treatment × time interaction, ε_{ijk} is the random residual error.

Differences among treatment means were tested for significance using Duncan's multiple range test. Effects were considered significant at a probability of $P \leq 0.05$ and $P \leq 0.01$.

Results and discussion

No statistically significant differences between the groups in the yield nor fat and protein content of milk were observed (Table 3). The highest milk yield during the trial period was noted in the case of group PrFO, while the highest protein content in milk of group FO+G. Lower somatic cells count was observed in milk of cows from group Protected fish oil when compared to other groups. An application of protected fish oil or fish oil with glycerol resulted in higher condition of cows when compared to the group fed with palm oil (Table 3).

Table 3
Milk yield, composition and somatic cells count, and condition of cows during the research period

	Protected palm oil	Protected fish oil	Fish oil and glycerol	SEM
Milk, kg	40.19	41.58	40.78	0.32
Fat, %	3.82	3.96	3.67	0.18
Protein, %	3.18	3.27	3.32	0.08
Lactose, %	0.97	0.96	0.97	0.09
Total solids, %	11.62	11.74	11.97	0.14
SCC, 1 000 cells/Ml	301.00^{a}	158.11^{ab}	346.78^{b}	32.9
BCS, 1 week of lactation	3.50^{a}	3.42^{b}	3.48^{a}	0.03
BCS, 4 week	3.32	3.29	3.35	0.15
BCS, 8 week	3.04^{a}	3.11^{b}	3.15^{b}	0.06

SCC: somatic cell count, BCS: body condition score, SEM: standard error of the mean, ^{a,b}significance of differences between groups at $P \leq 0.05$

Milk production, milk components and body weight were not significantly different among prilled fatty acids, calcium salts of long chain n-6 fatty acids or topdressed propylene glycol (Castañeda-Gutiérrez *et al.* 2009). In the present study, the highest milk yield was noted in cows that were given protected fish oil, however no statistically significant differences between the groups were observed. Another study demonstrated that cows fed with protected palm oil reached higher milk yield when compared to control cows or those fed with sunflower oil (Petit *et al.* 2004). Also the addition of calcium salts of long chain fatty acids caused an increase in milk yield in the first 12 weeks of lactation, accordingly with a decrease in milk protein content in the first 6 weeks of lactation (McNamara *et al.* 2003).

The effect of experimental diets on the concentrations of fatty acids in milk fat is presented in Table 4. No statistical differences in short-chain fatty acids (C:4 to C:12) content in milk fat were noted, however their lower value was observed in milk of cows from both groups where fish oil was used. Protected fish oil and fish oil administered with glycerol caused a decrease in medium-chain fatty acids content when compared to protected palm oil. The increase in long-chain acids was observed in milk fat of cows receiving protected fish oil when compared to the group PrPO. Fish oil protection, to a higher degree than administration of unprotected oil to cows, caused decrease in a concentration of saturated and unsaturated acids in milk fat (Table 4). In milk fat of cows from the group FO+G the content of *cis*-9, *cis*-12 C18:2 was higher when compared to the group PrPO. Concentration of *trans*-11 C18:1 was the highest in groups PrFO and FO+G, while the lowest in milk of cows receiving palm oil. The same relationships were observed in the case of *cis*-9, *trans*-11 CLA concentration in milk. After application of protected fish oil to cows, the content of CLA increased to 1.71 g/100 g of fatty acids, in milk

from group FO+G CLA content was only on slightly lower level. Observed *cis*-9, *trans*-11 CLA concentration was higher than in another studies (Shingfield *et al.* 2003, Osborne et. al. 2009) Higher content of EPA and DHA was also noted in milk fat in both experimental groups. DHA concentration in milk fat from control cows was below the detection threshold (10^{-3}).

Table 4
Fatty acid composition (g/100 g of total fatty acids) of milk from cows fed the experimental diets

Fatty acid	Protected palm oil	Protected fish oil	Fish oil and glycerol	SEM
C4:0	3.17^{A}	2.42^{B}	2.27^{B}	0.04
C6:0	1.99	1.64	1.50	0.21
C8:0	1.78	1.76	1.94	0.56
C10:0	2.50	2.36	2.39	0.34
C12:0	3.42	3.40	3.33	0.31
C14:0	10.92	10.36	10.27	0.45
C14:1	0.54	0.59	0.68	0.10
C16:0	35.25	30.20	30.19	0.82
C16:1	1.06	1.40	0.93	1.29
C18:0	12.19	10.24	10.01	0.68
C18:1 t9	0.19	0.36	0.39	0.27
C18:1 c9	18.33	16.96	17.20	1.80
C18:1 t11 (TVA)	1.11^{A}	3.67^{B}	2.90^{B}	0.87
C18:2 t9. t12	0.18^{A}	0.40^{B}	0.51^{B}	0.21
C18:2 c9. c12	2.95^{aA}	3.36^{b}	3.71^{B}	0.09
C18:2 c9. t11 (CLA)	0.61^{A}	1.71^{B}	1.68^{B}	0.24
C18:2 t9. t11 (CLA)	0.04^{A}	0.12^{B}	0.13^{B}	0.18
C18:3 n-3	0.24^{a}	0.32^{b}	0.31^{b}	0.19
C18:3 n-6	0.19^{A}	0.51^{B}	0.52^{B}	0.02
C20:1	0.11^{A}	1.56^{B}	1.34^{B}	0.39
C20:4 n-6	0.18^{a}	0.25^{b}	0.30^{b}	0.20
C20:5 n-3 (EPA)	0.03^{A}	0.22^{B}	0.24^{B}	0.01
C22:6 n-3 (DHA)	nd	0.23	0.21	0.08
Others	9.47	6.18	7.45	0.82
Short chain[1]	12.85	11.58	11.42	0.62
Medium chain[2]	47.77^{A}	42.56^{B}	42.09^{B}	0.97
Long chain[3]	36.35^{A}	46.00^{B}	39.30^{A}	1.34
Saturated	71.21^{A}	62.38^{B}	61.90^{B}	1.55
Unsaturated	25.76^{A}	31.58^{B}	30.90	1.73
CLA - desaturase index	0.25	0.27	0.28	0.10

TVA: trans vaccenic acid, CLA: conjugated linoleic acid, EPA: eicosapentaenoic acid, DHA: docohexaenoic acid, [1]Short chain fatty acids (SCFA): from C:4 to C:12, [2]Medium-chain fatty acids (MCFA) C:14-C:16, [3]Long-chain fatty acids (PUFA)>C:17, SEM, standard error of the mean, nd: not detected or detected at $P \leq 0.01$, ^{a,b}significance of differences between groups at $P \leq 0.05$, ^{A,B}significance of differences between groups at $P \leq 0.01$

The long-chain fatty acids of fish oil inhibit the final biohydrogenation step to 18:0, thereby maximizing yield of 18:1 trans intermediates (Shingfield *et al.* 2003, Loor *et al.* 2005). When using fish oil in cows feeding, a distinct increase in TVA in milk fat was noted (Donovan *et al.* 2000, AbuGhazaleh *et al.* 2004, Castañeda-Gutiérrez *et al.* 2007). Castañeda-Gutiérrez *et al.* (2007) after protected FISH OIL application noted the content of TVA on a level of 1.53-2.22 g/100 g of fatty acid, however that value was significantly higher (18.33 g/100 g of fatty acid) when fish oil was administered to cows in a form of ruminal infusion. It was noted in the

studies cited that the highest content of *cis*-9, *trans*-12 CLA in milk fat was observed when using fish oil in a form of ruminal infusion (6.05 g/100 g of fatty acid), and the lowest, 1.04 g/100 g of fatty acids, with high doses of protected fish oil. Supplementation of fish oil to TMR of cows caused higher increase in *cis*-9, *trans*-12 CLA content in milk fat when compared to its application with drinking water (Osborne *et al.* 2009). Donovan *et al.* (2000) observed, that an addition of 2 % of fish oil was the most effective, since caused an increase in *cis*-9, *trans*-11 CLA and TVA of 360 and 430 %, respectively. Similar results were obtained in another study after an application of fish oil on mineral carrier (Kupczyński *et al.* 2011). Supplementation of algae or fish oil with algae resulted in similar high concentration of CLA (AbuGhazaleh *et al.* 2009). In that study, the highest CLA content was noted when administering to cows 50 g of fish oil plus 100 g of algae per day.

Normally, DHA occurs only in trace amounts in milk fat of cows fed conventional diets. After an application of protected tuna oil, significant increase in n-3 acids in milk was noted, without deleterious effects on yield, milk composition or sensory characteristics (Kitessa *et al.* 2004). Similar results were also obtained in the present study in both groups of cows (PrFO and FO+G). In another study, after abomasal fish oil infusion, the content of DHA in milk fat was on a level of 0.63 g/100 g of fatty acids (Castañeda-Gutiérrez *et al.* 2007). In these studies, ruminal infusion or application of protected fish oil with feed caused lower DHA increase, 0.20 and 0.14 g/100 g total fatty acids, respectively. Also after an application of fish oil with marine algae, the content of DHA on a level of 0.06 g/100 g fatty acids was noted (AbuGhazaleh *et al.* 2009). Not in all the studies such a spectacular results were obtained (Cruz-Hernandez *et al.* 2007). An increase in EPA and DHA acids concentration was distint in the present study.

Mean values of selected serum lipid-carbohydrate parameters are shown in Table 5. Glucose concentration was higher in the group FO+G when compared to PrPO group. Similar high glucose concentration in blood was noted in the group PrFO. During the whole experiment BHBA concentration in blood did not exceed the normal range in all examined groups. The level of NEFA in blood serum tended to decrease in all groups in 4th week of the experiment. Cholesterol content was higher in the group PrFO when compared to PrPO group (Table 5). The application of protected fish oil caused an increase in TAG content to the references values (Meyer & Harvey 2004).

The highest increase in AST activity was noted in group PrPO. The activity of that enzyme in group FO+G was the lowest. The application of palm oil in cows caused a significant ($P \leq 0.01$) decrease in GGT activity when compared to fish oil and glycerol. The level of total bilirubin in blood serum of cows from all the groups was within the limits of reference values (Meyer & Harvey 2004), a small increase in its concentration was noted in group PrPO.

Table 5
Concentration of chosen biochemical parameters in blood serum

	Protected palm oil	Protected fish oil	Fish oil and glycerol	SEM
Glucose, mmol/l	3.25^{A}	3.50	3.48^{B}	0.12
BHBA, mmol/l	0.50	0.43	0.44	0.02
NEFA, mmol/l	0.61	0.41	0.57	2.10
Cholesterol, mmol/l	5.92	6.34^{A}	4.99^{B}	0.09
TAG, mmol/l	0.13^{A}	0.29^{B}	0.16^{A}	0.27
AST, U/l	89.84	88.49	79.02	0.78
GGT, U/l	39.31^{A}	34.16	32.48^{B}	0.46
Total bilirubin, mmol/l	6.10	5.07	4.52	1.30

BHBA: β-hydroxybutyrate acid, NEFA: nonesterified fatty acids, TAG: triacylglycerols, AST: aspartate aminotransferase, GGT: γ-glutamylotransferase, SEM: standard error of the mean, ^{A,B}significance of differences between groups at $P \leq 0.01$

Different influence of protected fat application on metabolism and reproduction of cows may result from the content of fatty acids in that supplements (McNamara *et al.* 2003). An application of rumen-protected fat in cows feeding caused the decrease in glucose concentration in blood (Drackley *et al.* 2003, Hammon *et al.* 2008). In the study by Hammon *et al.* (2008) however, an increase in lactose yield after rumen-protected fat application was observed. The authors concluded that the glucose sparing-effect allowed an enhanced lactose synthesis when feeding rumen-protected fat. In the present study, protected fish oil containing large amounts of PUFA caused a linear increase in glucose concentration in blood serum of cows. The tendency of blood NEFA decrease was noted after an application of protected fish oil. In the study by Petit *et al.* (2004) an application of protected palm oil did not influence statistically the NEFA, glucose, cholesterol and HDL and LDL level.

Using calcium salts of long chain n-6 fatty acids (CaLFA), prilled fat and propylene glycol in a transition period, higher yield and more profitable energy balance was observed in cows that were given supplements, when compared to control cows (Castañeda-Gutiérrez *et al.* 2009). The addition of protected fats and propylene glycol contributed in a decrease in BHBA after calving (Castañeda-Gutiérrez *et al.* 2009). The application of CaLFA with high content of unsaturated acids during the first 100 days of lactation caused the increase of NEFA in blood in comparison to prilled fat application (Moallem *et al.* 2007). In another study, the fish oil (unprotected) did not influence the blood NEFA concentration, causing the decrease in glucose level (Mattos *et al.* 2004). Fish oil increased the proportion of individual and total n-3 fatty acids (linolenic, EPA, and DHA) in milk.

The application of powdered glycerol in transition period improved the energy status of cows by higher concentrations of plasma glucose, lower concentrations of plasma BHBA, and lower concentration of urine ketones (DeFrain *et al.* 2005). This glucogenic effect of dry glycerol did not result in statistically significant increase in feed intake or milk yield during the first 3 weeks of lactation. Fish oil and glycerol given to cows in TMR dose in the present study caused a successive increase in blood glucose concentration. Glycerol administered orally for the first two weeks postpartum caused an increase in blood glucagon, NEFA and and BHBA levels in 7th and 13th day (Osman *et al.* 2008). More intense, profitable influence on metabolism was observed in the case of a joint subcutaneous application of glycerol and glucagon injection. Glucogenic activity of glicerol was not confirmed in the study by DeFrain *et al.* (2004) and Osborne *et al.* (2009). Patton *et al.* (2004) giving glucogenic precursors

(propylene glycol and calcium propionate) together with protected fat (CaLFA) in a transition period observed a synergistic activity consisted in an increase in glucose and insulin level and decrease in NEFA level in blood.

Finally, when using protected palm oil, protected fish oil or unprotected fish oil with glycerol in TMR, no differences in yield and milk composition were noted between the experimental groups. Fatty acids content was clearly influenced by increased PUFA content in feed doses where fish oil supplementation was used. In milk fat of cows receiving fish oil, significant ($P \leq 0.01$) increase in long-chain acids content was observed when compared to the group receiving protected palm oil. Fish oil protection led to the highest degree of saturated acids concentration lowering in milk fat. The concentration of *cis*-9, *trans*-11 CLA, TVA and EPA/DHA in milk fat increased when protected fish oil and fish oil and glycerol were applied. These results indicate that introduction of PUFA to cows diet may clearly influence fatty acids concentration in milk fat. An application of fish oil with an addition of glucogenic precursor and protected fish oil exhibit a positive influence on glucose homeostasis at the beginning of cows lactation.

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References

AbuGhazaleh AA, Schingoethe DJ, Hippen AR, Kalscheur KF (2004) Conjugated linoleic acid increases in milk when cows fed fish meal and extruded soybeans for an extended period of time. J Dairy Sci 87, 1758-1766

AbuGhazaleh AA, Felton DO, Ibrahim SA (2007) Milk conjugated linoleic acid response to fish oil and sunflower oil supplementation to dairy cows managed under two feeding systems. J Dairy Sci 90, 4763-4769

AbuGhazaleh AA, Potu RB, Ibrahim S (2009) The effect of substituting fish oil in dairy cow diets with docosahexaenoic acid-micro algae on milk composition and fatty acids profile. J Dairy Sci 92, 6156-6159

AOAC (Association of Official Analytical Chemists): Official Methods of Analysis. 18th ed. AOAC International, Arlington, VA, 2005

Avramoglu RK, Adeli K (2004) Hepatic regulation of apolipoprotein B. Rev Endocr Metab Disord 5, 293-301

Castañeda-Gutiérrez E, de Veth MJ, Lock AL, Dwyer DA, Murphy KD, Bauman DE (2007) Effect of supplementation with calcium salts of fish oil on n-3 fatty acids in milk fat. J Dairy Sci 90, 4149-4156

Castañeda-Gutiérrez E, Pelton SH, Gilbert RO, Butler WR (2009) Effect of peripartum dietary energy supplementation of dairy cows on metabolites, liver function and reproductive variables. Anim Reprod Sci, 112, 301-315

Chilliard Y, Ferlay A, Doreau M (2001) Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest Prod Sci 70, 31-48

Chouinard PY, Corneau L, Butler WR, Bauman DE, Chilliard Y, Drackley JK (2001) Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J Dairy Sci 84, 680-690

Cruz-Hernandez C, Kramer JKG, Kennelly JJ, Glimm DR, Sorensen BM, Okine EK, Goonewardene LA, Weselake RJ (2007) Evaluating the conjugated linoleic acid and trans 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. J Dairy Sci 90, 3786-3801

Deeth HC, Fitzgerald CH, Snow AJ (1983) A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. New Zealand J Dairy Sci Technol 18, 230-233

DeFrain JM, Hippen AR, Kalscheur KF, Jardon PW (2004) Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. J Dairy Sci 87, 4195-4206

DeFrain JM, Hippen AR, Kalscheur KF, Patton RS (2005) Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J Dairy Sci 88, 983-993

Donovan DC, Schingoethe DJ, Baer RJ, Ryali J, Hippen AR, Franklin ST (2000) Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J Dairy Sci 83, 2620-2628

Drackley JK, Cicela TM, LaCount DW (2003) Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. J Dairy Sci 86, 1306-1314

Garnsworthy PC, Lock A, Mann GE, Sinclair KD, Webb R (2008) Nutrition, metabolism, and fertility in dairy cows: 2. Dietary fatty acids and ovarian function. J Dairy Sci 91, 3824-3833

Grummer RR (2008) Nutritional and management strategies for the prevention of fatty liver in dairy cattle. Vet J 176, 10-20

Hammon HM, Metges CC, Junghans P, Becker F, Bellmann O, Schneider F, Nürnberg G, Dubreuil P, Lapierre H (2008) Metabolic changes and net portal flux in dairy cows fed a ration containing rumen-protected fat as compared to a control diet. J Dairy Sci 91, 208-217

Hayirli A, Grummer RR, Nordheim EV, Crump PM (2002) Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J Dairy Sci 85, 3430-3443

Heravi Moussavi AR, Gilbert RO, Overton TR, Bauman DE, Butler WR (2007) Effects of feeding fish meal and n-3 fatty acids on milk yield and metabolic responses in early lactating dairy cows. J Dairy Sci 90, 136-144

Kitessa SM, Gulati SK, Simos GC, Ashes JR, Scott TW, Fleck E, Wynn PC (2004) Supplementation of grazing dairy cows with rumen-protected tuna oil enriches milk fat with n-3 fatty acids without affecting milk production or sensory characteristics. Br J Nutr 91, 271-277

Kupczyński R, Szołtysik M, Janeczek W, Chrzanowska J, Kinal S, Króliczewska B (2011) Effect of dietary fish oil on milk yield, fatty acids content and serum metabolic profile in dairy cows. J Anim Physiol Anim Nutr 95, 512-522

Loor JJ, Ferlay A, Ollier A, Ueda K, Doreau M, Chilliard Y (2005) High-concentrate diets and polyunsaturated oils alter trans and conjugated isomers in bovine rumen, blood, and milk. J Dairy Sci 88, 3986-3999

Mattos R, Staples CR, Arteche A, Wiltbank MC, Diaz FJ, Jenkins TC, Thatcher WW (2004) The effects of feeding fish oil on uterine secretion of PGF2α, milk composition, and metabolic status of periparturient Holstein cows. J Dairy Sci 87, 921-932

McNamara S, Butler T, Ryan DP, Mee JF, Dillon P, O'Mara FP, Butler ST, Anglesey D, Rath M, Murphy JJ (2003) Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving Holstein–Friesian cows. Anim Reprod Sci 79, 45-56

Meyer DJ, Harvey JW (2004) Veterinary laboratory medicine: interpretation and diagnosis. 3rd ed. W.B. Saunders Company. St. Louis, USA

Moallem U, Katz M, Arieli A, Lehrer H (2007) Effects of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake, production, and plasma metabolites in dairy cows. J Dairy Sci 90, 3846-3856

Osborne VR, Odongo NE, Cant JP, Swanson KC, McBride BW (2009) Effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient dairy cows. J Dairy Sci 92, 698-707

Osman MA, Allen PS, Mehyar NA, Bobe G, Coetzee JF, Koehler KJ, Beitz DC (2008) Acute metabolic responses of postpartal dairy cows to subcutaneous glucagon injections, oral glycerol, or both. J Dairy Sci 91, 3311-3322

Overton TR, Waldron MR (2004) Nutritional management of transition dairy cows: strategies to optimize metabolic health. J Dairy Sci 87 (E suppl.), 105-119

Patton RS, Sorenson CE, Hippen AR (2004) Effects of dietary glucogenic precursors and fat on feed intake and carbohydrate status of transition dairy cows. J Dairy Sci 87, 2122-2129

Petit HV, Germiquet C, Lebel D (2004) Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. J Dairy Sci 87, 3889-3898

Piperova LS, Sampugna J, Teter BB, Kalscheur KF, Yurawecz MP, Ku Y, Morehouse KM, Erdman RA (2002) Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. J Nutr 132, 1235-1241

Shingfield KJ, Ahvenjärvi S, Toivonen V, Ärölä A, Nurmela KVV, Huhtanen P, Griinari JM (2003) Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Animal Science 77, 165-179

Shingfield KJ, Reynolds CK, Hervás G, Griinari JM, Grandison AS, Beever DE (2006) Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J Dairy Sci 89, 714-732

van Knegsel ATM, van den Brand H, Dijkstra J, van Straalen WM, Jorritsma R, Tamminga S, Kemp B (2007) Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. J Dairy Sci 90, 3397-3409

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