# INFLUENCE OF FISH OIL LONG-TERM ADDITION ON FATTY ACIDS CONTENT IN MILK OF DAIRY COWS

**Kupczyński, R., Janeczek, W., Szołtysik, M.*, Pogoda-Sewerniak, K. and Korczyński, M.**

*Wroclaw University of Environmental and Life Sciences, Department of Animal Hygiene and Environment, ul. Chełmonskiego 38c, 51–630 Wroclaw, Poland, * Department of Animal Products Technology, Wroclaw University of Environmental and Life Sciences, Poland*

## SUMMARY

The aim of investigation was to evaluate of long-term addition of fish oil effect on fatty acids contents in milk fat. The cows were administered preparation containing fish oil (1% in DM) which resulted in decreased level of short chain fatty acids in milk fat and increased level of long chain fatty acid. The addition of mineral-fat preparation had a significant effect on growth of *cis*-9, *trans*-11 CLA content as well as transvaccenic acid and omega-3 fatty acids (EPA, DHA) in milk fat of cows. The growth of CLA was 364.8% in primiparous milk and 454.8% in multiparous milk, respectively.

**Keywords:** dairy cows, fish oil, fatty acids, conjugated linoleic acid, transvaccenic acid

## INTRODUCTION

Milk fat contains several compounds with specific biological properties. One of these is conjugated linoleic acid (CLA). In animal models, CLA has been shown to inhibit the growth of cancer, exhibit anti-atherogenic effects, and alter body and bone metabolism [10, 15, 17]. Based in part on these observations, CLA is perceived to be a dietary constituent that may have potential benefit as a modulator of human disease.

CLA content in milk fat is determined by such factors as, e.g. breed, age, stage of lactation. The strongest effects belong to food factors [1, 5, 7, 12, 13, 14, 20]. Supplementation of food rations for cows with mono- and poly- unsaturated fatty acids in the form of seeds, extruded crushed meal, calcium oils and salts influences PUFA biohydrogenation in rumen content, modifying milk composition [2,3, 5, 7]. Linoleic and linolenic fatty acids supplied with forage undergo biohydrogenation in rumen due to *Butyrivibrio fibrisolvents* and other bacteria to stearic acid. In those alterations *cis*-9,*trans*-11 CLA isomer and vaccenic acid (VA, *trans*-11 $C_{18:1}$) are intermediate products [5, 14]. *Cis*-9,*trans*-11 CLA present in milk fat is synthesized in 64-78% in milk gland with VA by $\Delta^9$-desaturase (stearoyl-CoA desaturase) [5, 8, 16]. *Cis*-9, *trans*-11 CLA synthesis in milk gland can range up to 91% of total CLA milk fat content [12]. Increasing VA is therefore an important part of this research.

Feeding lipid sources rich in mono- and polyunsaturated fatty acids, either as seeds, free oil, or calcium salts, increased the *cis*-9, *trans*-11 CLA and VA content of milk when oil is accessible to the rumen microorganisms for biohydrogenation. In previous studies [2, 18, 20] fat supplements were fed for not more than 4 wk. AbuGhazaleh et al. [1] used the simultaneous supplementation of 0.5% fish oil from fish meal and 2% soybean oil from extruded soybeans. Relatively high

growth of cis-9, trans-11 CLA in milk was apparent through first 5 weeks (in 3 weeks was 350%), and next content of these isomers slightly decreased.

The aim of this work was estimation of effect of fish oil long-term addition (on mineral support) in feeding of high productive dairy cows onto fatty acids content, with specially regard of *cis*-9, *trans*-11 CLA and n-3 polyunsaturated fatty acids (PUFA n-3).

## MATERIAL AND METHODS

The experiment was executed in milk cows' farm with stock of 280 cows and with mean yield of 8200 kg of milk yearly. Experimental cows were kept in the same environmental conditions and they were fed of TMR (total mixed rations). The fodders used for cow feeding (TMR components) were subjected to chemical analysis in Blattin Laboratory in Langenfeld to determine the content of dry mass, total protein, raw ash, raw fat, raw fibre and its fractions (ADF and NDF), as well as mineral components: calcium, phosphorus, magnesium and sodium. On the basis of the analysis done, there were worked out food rations for cows according to DLG norms. Quantitative composition and food value of the doses were shown in Table 1. There were 40 cows in these studies, which were divided onto subsequent groups (n=10):

group I – control group (primiparous ),
group II –primiparous, fed with 1% of fish oil in DM,
group III –control group (multiparous in 2 or 3 lactation),
group IV – multiparous (in 2 or 3 lactation), fed with 1% of fish oil in DM.

The fish oil was mixed with mineral component, additionally administered for 8 weeks twice a day in the dose of 563 g per head (1126 per day). Obtained mineral-fat preparation contained: fish oil (herring-sprat) – 25%, baidelit – 33%, wernikulit – 33% and humokarbowit – 9%. The preparation was added beginning from 1 week of lactation during 8 subsequent weeks. Composition of fatty acids from fish oil was marked by chromatographic method. The results of these analyses have been showed in other research [11].

The representative milk samples were taken from cows in day of beginning of research (1 week of lactation) and in day of ending up of research (after 8 weeks of FO addition). Fatty acid composition was determined in representative samples following gas chromatography method with the use of gas chromatograph Agilent Technologies 5973. Separation was carried out in the following conditions: column 60m x 0.25μm, column temperature ranged 140 °C (5 min) to 240 °C (4 °C/min), carrier gas – helium (20 m/s), spray 1 μl, 260 °C, split 100:1.

The values obtained were statistically worked out using statistical program Statgraphics Version 5.0 and difference significance was estimated according to Duncan test.

## RESULTS AND DISSCUSION

The assessment of fatty acid contents in fish oil showed that it features high percentage of polyunsaturated fatty acids (PUFA) – 40.43% including linoleic acid (LA) 7.28%, eicosapentaenoic (EPA) 8.19% and docoshexaenoic acids (DHA) 13.83% [11]. In Poland the fish oils are obtained mainly from herrings, sprats a mackerels. American investigation involving cow food made use of fish oil originating from menhadan. That oil contained less EPA and DHA – 10.93 and 11.92 g / 100 g fatty acids respectively than fish oil applied in our own investigation

[2,3], while the content of polyunsaturated fatty acids was lower than the one in our own investigation.

Using of fatty-mineral preparation, which contained the fish oil (1% of DM) as an addition to TMR dose for cows caused decreasing of content of short-chain fatty acids in milk (C 4:0 – C12:0) and growth of long-chain content (C 16:1 – C22:6).

In day of beginning the study, concentration of *cis*-9, *trans*-11 CLA isomers were on similar level in individual groups (0.54 g/100g of fatty acids in groups I and III; and 0.64 g/100g of fatty acids II and IV ). In day of the investigations finish, content of cis-9, trans-11 CLA were 1.97 (primiparous, group II) and 2.82 g/100g of fatty acids (multiparous, group IV). The growth was 364.8% in primiparous milk and 454.8% in multiparous milk, respectively. The significant growth of vaccenic acid concentration in milk, 387.8% (group II) and 255,9% (group IV) was found, respectively to 3.8 and 3.71 g/100g of fatty acids (tab. 2).

Although dietary fish oil dramatically and consistently increases milk VA and CLA concentrations, it can also decrease feed intake, milk production, and milk fat yield or concentration [6, 9, 20]. Donovan et al. [9] fed fish oil to dairy cows at 0, 1, 2, or 3% of diet DM and observed maximum concentrations of milk VA and CLA at 2% fish oil supplementation. Because fish oil contains low amounts of known precursors of VA and CLA, the authors speculated that fish oil enhanced the conversion of linoleic acid or linolenic acid, or both, from other feed sources into VA and CLA, possibly by inhibiting the final step in the biohydrogenation of VA to stearic acid [18]. Whitlock et al. [20] found that milk VA and CLA were increased similarly for cows fed 1% fish oil in combination with 1% fat from extruded soybeans compared with when cows were fed 2% fish oil. When cows were fed a diet containing 2% fat from extruded soybeans, VA and CLA concentrations increased less than half as much as when they were fed the fish oil-supplemented diets.

When fish oil and sunflower seeds (linoleic acid source) were fed, milk concentrations of *cis*-9, *trans*-11 CLA and VA averaged 1.7 and 3.74 g/100 g of fatty acids, respectively [2]. Milk fat from cows fed a control diet (no added fat) contains approximately 0.4 and 0.75 g/100 g fatty acids of *cis*-9, *trans*-11 CLA and VA [3]; therefore, feeding a blend of fish oil and unsaturated fat source such as extruded soybeans or sunflower seeds increased milk *cis*-9, *trans*-11 CLA and VA by 300 to 400%, respectively. Milk fat *cis*-9, *trans*-11 CLA and VA concentration (g/100 of fatty acids) and yield (g/d) were 2.5-fold greater for cows fed the fish meal and extruded soybeans diet over the 10 wk of fat supplementation [1]. In own investigation used mineral-fat preparation caused the growth of CLA In milk until to 450%. This kind connection of fish oil at long-term supplementation she was (8 weeks) very effective. Shingfield et al. [19] reported that administration of fish oil with sunflower oil resulted (on the fifth day) in higher *cis*-9, *trans*-11 CLA concentration up to 5.37g/100g fatty acids, while after 15 days of using the mentioned supplementation, concentration of *cis*-9, *trans*-11 CLA decreased to 2.35g/100g fatty acids.

Some authors particularly show that in TMR cow feeding system CLA value in milk remains lower in comparison to the milk collected from the cows fed on a pasture [6, 5, 12]. Therefore, our own investigation confirms purposefulness of the described supplementation of food rations, especially in the situation of common use of TMR system on highly efficient cow farms.

The fish oil, added to the diet caused growth of omega-3-polyunsaturated acids (EPA and DHA) content in milk, and it was higher in milk of multiparous cows. Mineral-fat preparation caused the growth of DHA after 8 weeks supplementation, to 0.18 and 0.31 g/100g of fatty acids in groups II and IV, respectively. That fact was also confirmed by other investigations [2, 3, 20] which proved that EPA and DHA concentration in cow milk fat did considerably increase after application of vegetable oils with fish oil. Generally, EPA and DHA transfer from food ration to

milk is low [1] since they are preferentially deposited in body tissues rather than in milk fat. The results obtained confirm purposefulness of fish oil supplementation in cow food rations which is also reflected in milk nutritional value in human diet.

## CONCLUSIONS

The addition of mineral-fat preparation had a significant effect on growth of *cis*-9, *trans*-11 CLA content as well as transvaccenic acid and omega-3 fatty acids (EPA, DHA) in milk fat of cows. The growth of CLA was 364.8% in primiparous milk and 454.8% in multiparous milk, respectively.

## ACKNOWLEDGMENTS

The research was granted by Polish Ministry of Science and Higher Education, grant No. 2 P06Z 060 27.

## REFERENCES

1. AbuGhazaleh A.A., Schingoethe D.J., Hippen A.R., Kalscheur K.F., 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.
2. Abu-Ghazaleh A.A., Schingoethe D.J., Hippen A.R., Kalscheur K.F., 2003: Milk conjugated linoleic acid response to fish supplementation of diets differing in fatty acid profiles. J. Dairy Sci. 86 (3): 944–953.
3. Abu-Ghazaleh A.A., Schingoethe D.J., Hippen A.R., Whitlock L.A., 2002. Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. J. Dairy Sci. 85 (3): 624–631.
4. Bakuła T., Apoznański J., Obremski K., Iwaniuk Z., Gajęcki M., 2005. Skład chemiczny wybranych rybnych materiałów paszowych. Medycyna Wet. 61 (9): 1061–1063.
5. Bauman D. E., Baumgard L. H., Corl B. A., Griinari J. M. 2000. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci. Available at: www.asas.org/jas/symposia/proceedings/0937.pdf.
6. 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.
7. Chouinard P. Y., Corneau L., Butler W. R., Chilliard Y., Drackley J. K., Bauman D. E., 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J. Dairy Sci. 84: 680–690.
8. Corl B.A., Baumgard L.H., Dwyer D.A., Griinari J.M., Phillips B.S., Bauman D.E., 2001. The role of 9-desaturase in the production of *cis*-9, *trans*-11 CLA. J. Nutr. Biochem. 12: 622–630.
9. Donovan D.C., 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. J. Dairy Sci. 83 (11): 2620–2628.
10. Ip C., Banni S., Angioni E., Carta G., McGinley J., Thompson H. J., Barbano D., Bauman D. 1999. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J. Nutr. 129, 2135–2142.
11. Janeczek W. Szołtysik M., Kinal S., Pogoda-Sewerniak K., Kupczyński R.: Influence of fish oil addition on fatty acids content in milk of dairy cows. Chemistry for Agricylture. Vol. 7, 2006, 877–882.
12. Kay J.K., Mackle T.R., Auldist M.J., Thomsan N.A., Bauman D.E., 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87: 369–378.

13. Kelly, M.L., J.R. Berry, D.A. Dwyer, J.M. Griinari, P.Y. Chouinard, M.E. Van Amburgh and D.E. Bauman, 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating airy cows. J. Nutr. 128: 881–885.
14. Loor, J.J., 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.
15. Park Y., Albright W.L., Storkson J.M., Cook M.E., Pariza M.W. 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids. 32, 853– 858.
16. Piperova, L.S., Sampugna J., Teter B.B., Kalscheur K.F., Yurawecz M.P., Ku Y., Morehouse K.M., Erdman R.A., 2002. Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate thatpostabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. J. Nutr.132: 1235–1241.
17. Roche H.M., Noone E., Nugent A., Gibney M.J., 2001. Conjugated linoleic acid: a novel therapeutic nutrient? Nutr. Res. Rev. 14: 173–187.
18. Shingfield K., Ahvenjärvi S., Toivonen V., Ärölä A., Nurmela K.V.V., Huhtanen P., Griinari J. 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim. Sci. 77: 165–179.
19. Shingfield K.J. Reynolds C.K., Hervas G., Griinari J.M., Grandison A.S., Beever D.E., 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.
20. 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. J. Dairy Sci. 85: 234–243.

**Table 1**. Content and nutritional composition of the cows' diets

| Dose composition | Units | | |
|---|---|---|---|
| Maize silage | kg | 25,000 | |
| Fresh pressed pulp | | 8,000 | |
| Maize seed silage | | 5,500 | |
| Barley | | 3,000 | |
| Soybean | | 2,700 | |
| Rapeseed | | 2,500 | |
| Barley strain | | 2,000 | |
| Sodium bicarbonate | | 0,200 | |
| Premix | | 0,180 | |
| Forage chalk | | 0,150 | |
| Fat-mineral preparation* | | 1,125 | |
| Nutritional composition | | Control groups I and III – (primiparous and multiparous) | Experimental groups II and IV (primiparous and multiparous) |
| Dry mass | % | 48,17 | 49,06 |
| Raw fibre % dry mass | % s.m. | 14,03 | 13,65 |
| NEL | MJ/kg s.m. | 6,87 | 8,24 |
| Total protein | g/kg s.m. | 3858,00 | 3864,59 |
| Available total protein in small intensive | g/kg s.m. | 3806,63 | 3813,19 |
| Ca | g/kg s.m. | 6,95 | 6,75 |
| P | g/kg s.m. | 4,36 | 4,35 |
| Na | g/kg s.m. | 1,40 | 1,35 |
| Mg | g/kg s.m. | 2,54 | 3,19 |

*preparation supplementation (containing fish oil) in experimental groups (II and IV)

**Table 2**. Fatty acids composition in milk fat of cows (g/100g fatty acids)

| Fatty acids | Group I – control | | Group II – experimental | | Group III – control | | Group IV – experimental | |
|---|---|---|---|---|---|---|---|---|
| | Period of T-M preparation administration | | | | | | | |
| | 0 | 8th week | 0 | 8th week | 0 | 8th week | 0 | 8th week |
| $C_{4:0}$ | 2,89 | 2,72 | 2,89 | 2,54 | 3,14 $^{A}$ | 3,25 $^{A}$ | 3,14 $^{A}$ | 1,99$^{B}$ |
| $C_{6:0}$ | 1,25$^{A}$ | 1,53$^{B}$ | 1,25 $^{A}$ | 1,00 $^{B}$ | 2,34 $^{A}$ | 2,31 $^{B}$ | 2,34 $^{A}$ | 1,82 $^{B}$ |
| $C_{8:0}$ | 1,73 | 1,62 | 1,73 $^{A}$ | 0,93 $^{B}$ | 1,82 $^{A}$ | 1,73 $^{A}$ | 1,82 $^{A}$ | 1,31 $^{B}$ |
| $C_{10:0}$ | 2,36 | 2,33 | 2,36 $^{A}$ | 1,82 $^{B}$ | 2,84 $^{A}$ | 2,6 2 $^{B}$ | 2,84 $^{A}$ | 2,01 $^{B}$ |
| $C_{12:0}$ | 3,01 $^{A}$ | 3,37 $^{B}$ | 3,01 $^{A}$ | 3,11 $^{B}$ | 3,56 | 3,41 | 3,56 | 3,54 |
| $C_{14:0}$ | 11,83 $^{A}$ | 10,52 $^{B}$ | 11,83 $^{A}$ | 9,97 $^{B}$ | 11,93 $^{A}$ | 10,58 $^{B}$ | 11,93 $^{A}$ | 10,21 $^{B}$ |
| $C_{14:1}$ | 0,56 $^{A}$ | 0,51 $^{A}$ | 0,56 $^{A}$ | 0,44 $^{B}$ | 0,62 $^{A}$ | 0,59 $^{B}$ | 0,62 $^{A}$ | 0,47 $^{B}$ |
| $C_{16:0}$ | 29,13 $^{A}$ | 30,12 $^{B}$ | 29,13 $^{A}$ | 30,62 $^{B}$ | 30,24 | 30,50 | 30,24 | 31,02 |
| $C_{16:1}$ | 1,02 | 1,01 | 1,02 $^{A}$ | 2,28 $^{B}$ | 1,24 $^{A}$ | 1,04 $^{B}$ | 1,24 $^{A}$ | 2,99 $^{B}$ |
| $C_{18:0}$ | 11,40 $^{A}$ | 12,41 $^{B}$ | 11,40 $^{A}$ | 10,22 $^{B}$ | 12,03 | 12,56 | 12,03 $^{A}$ | 11,03 $^{B}$ |
| $C_{18:1t9}$ | 0,14 | 0,15 | 0,14 $^{A}$ | 0,25 $^{B}$ | 0,23 | 0,20 | 0,23 $^{A}$ | 0,34 $^{B}$ |
| $C_{18:1c9}$ | 18,36 | 18,8 | 18,36 | 16,83 | 17,45 | 18,36 | 17,45 | 16,01 |
| $C_{18:1t11\ (TVA)}$ | 0,98$^{A}$ | 0,99 $^{A}$ | 0,98 $^{A}$ | 3,80$^{B}$ | 1,45 $^{A}$ | 1,12 $^{A}$ | 1,45 $^{A}$ | 3,71$^{B}$ |
| $C_{18:2t9,t12}$ | 0,21 $^{A}$ | 0,23 | 0,21 $^{A}$ | 0,33$^{B}$ | 0,17 $^{A}$ | 0,15 $^{A}$ | 0,17 $^{A}$ | 0,40$^{B}$ |
| $C_{18:2c9,c12}$ | 2,78 $^{A}$ | 2,79 $^{A}$ | 2,78 $^{A}$ | 1,63$^{B}$ | 3,11 $^{A}$ | 3,09 $^{A}$ | 3,11 $^{A}$ | 1,89$^{B}$ |
| $C_{18:2c9,t11(CLA)}$ | 0,54$^{A}$ | 0,63$^{B}$ | 0,54$^{A}$ | 1,97$^{C}$ | 0,62$^{A}$ | 0,60$^{A}$ | 0,62$^{A}$ | 2,82$^{B}$ |
| $C_{18:2t9,t11(CLA)}$ | 0,02 | 0,03 | 0,02 | 0,08 | 0,05$^{A}$ | 0,06$^{A}$ | 0,05$^{A}$ | 0,18$^{B}$ |
| $C_{18:3\ n-3}$ | 0,04 | 0,05 | 0.04 | 0,06 | 0,03 | 0,09 | 0.03 $^{A}$ | 0,20$^{B}$ |
| $C_{18:3\ n-6}$ | 0,16 | 0,18 | 0,16 | 0,61 | 0,17 | 0,22 | 0,17 | 0,80 |
| $C_{20:1}$ | 0,08 | 0,10 | 0,08 | 2,42 | 0,07 | 0,15 | 0,07 | 2,95 |
| $C_{20:4\ n-6}$ | 0,16 | 0,16 | 0,16 | 0,27 | 0,18 | 0,19 | 0,18 | 0,31 |
| $C_{20:5\ n-3\ (EPA)}$ | 0.03$^{A}$ | 0,03$^{A}$ | 0.03$^{A}$ | 0,40$^{B}$ | 0.02$^{A}$ | 0,02 $^{A}$ | 0.02 $^{A}$ | 0,46$^{B}$ |
| $C_{22:6\ n-3\ (DHA)}$ | – | – | – | 0,18 | – | – | – | 0,31 |
| Σ | 85,46 | 90,28 | 88,68 | 91,57 | 93,31 | 90,02 | 93,31 | 96,77 |
| Other | 14,54 | 9,72 | 11,32 | 8,43 | 6,69 | 9,98 | 6,69 | 3,23 |
| Short-chain[1] | 11,24 | 11,57 | 11,24 | 9,4 | 13,7 | 10,07 | 13,7 | 10,67 |
| Medium-chain [2] | 42,54 | 42,16 | 42,54 | 43,31 | 44,03 | 42,71 | 44,03 | 44,69 |
| Long-chain [3] | 34,76 | 36,55 | 34,9 | 39,05 | 35,58 | 36,61 | 35,58 | 41,41 |
| Saturated | 65,18 | 64,62 | 65,18 | 60,21 | 69,76 | 65,97 | 69,76 | 62,93 |
| Unsaturated | 24,94 | 25,66 | 25,08 | 31,55 | 25,41 | 24,05 | 25,41 | 33,84 |
| ∑ EPA, DHA | 0,03 | 0,03 | 0,03 | 0,58 | 0,02 | 0,02 | 0,02 | 0,77 |
| CLA*/ TVA | 0,55 | 0,64 | 0,55 | 0,52 | 0,43 | 0,54 | 0,43 | 0,76 |
| n-6/ n-3 | 4,57 | 4,25 | 4,57 | 1,38 | 7,00 | 3,73 | 7,00 | 1,14 |

TVA – vaccenic acid, CLA – conjugated linolic acid, EPA – eicosapentaenoic acid, DHA – docohexaenoic acid

[1] – short-chain fatty acids (C4:0 – C12:0); [2] – medium-chain fatty acids (C14:0 – C16:1); [3] – ling-chain fatty acids (> C16:0)

* $C_{18:2c9,t11}$ CLA

A,B – significant differences ($p<0.01$)