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EFFECT OF DIETARY SUPPLEMENTATION WITH PUFA n-3 ON THE LIPIDS COMPOSITION OF CHICKEN MEAT

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This paper investigates the possibilities of enrichment of meat with PUFA n-3, especially with EPA and DHA. For this purpose 225 Ross 208 male broilers were divided into 5 groups, each consisting of 45 broilers. From 1st to 21st day of the fattening period broilers were fed with standard diets that contained 22.67% crude protein and 14.19 MJ kg^{-1} ME. From 22nd to 42nd day broilers were fed with finisher diets, balanced at 20.43% crude protein and 14.18 MJ kg^{-1}. The first group was given F_1 diets, which, besides other feedstuffs contained 5% poultry fat; 2nd, 3rd, 4th and 5th group were fed with F_2, F_3, F_4, and F_5 diets, which were enriched with marine oil Pronova Biocare Epax 3000 TG in the amount of 0.5%, 1%, 1.5% and 2%, respectively. The obtained research results showed that PUFA n-3 was enhanced from 6% to 11.46% in the lipids of breast muscles, and from 3.71% to 10.44% in the lipids of thigh muscles. PUFA n-6/PUFA n-3 ratio was lowered from 2.95 to 1.22 in the breast muscles, and 5.35 to 1.60 in the thigh muscles.

Keywords: chicken meat, fatty acids, PUFA n-3

Changing the profile of fatty acids in the lipids of broiler meat with the aim to increase the content of polyunsaturated n-3 fatty acids (PUFA n-3) presents valuable scientific achievement in preserving the human health, especially in prevention of number of diseases, including coronary heart disease, cancer, diabetes and depression (LEAF & WEBER, 1988; BARLOW & PIKE, 1991; ALBRECHT & KLEIN, 1995; OKUYAMA & IKEMOTO, 1999). Vegetable oils (rape oil, linseed oil) rich in α-linolenic acid, as well as fish flour and marine oil (because of the high content of eicosapenthaenoic – EPH and docosahexaenoic – DHA acids) are used in order to increase the content of PUFA n-3 in the broiler meat (HULAN et al., 1984; CHANMUGAM et al., 1992; SCAIFE et al., 1994). Researches of many authors (HRDINKA et al., 1996; KRALIK et al., 1997; 2002; ZOLLITSCH et al., 1997; SANZ et al., 1999; KRASICKA et al., 2000; ZELENKA et al., 2001) prove that the food composition, particularly the content of fatty acids affects the

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profile of fatty acids in the lipids of broiler meat. Therefore, the aim of this research was to enrich the chicken meat with PUFA n-3 and to enhance the content of EPH and DHA by adding marine oil into the finisher diets that are used for fattening purposes.

1. Materials and methods

Two hundred twenty-five one-day-old Ross 208 male broilers were divided into five groups, each consisting of 45 chickens. Chickens in each group were fed from 1st to 21st day with starter mixture that contained 22.67% crude proteins and 14.19 MJ kg^{-1} ME (metabolizable energy). From 22nd to 42nd day chickens were fed with finisher diets, as follows: 1st group was given F_1 diets, which, besides other feedstuffs contained 5% poultry fat; 2nd, 3rd, 4th and 5th group were fed with F_2, F_3, F_4, and F_5 diets, which were enriched with marine oil Pronova Biocare Epax 3000 TG (PBE) in the amount of 0.5%, 1%, 1.5% and 2%, respectively. At the same time poultry fat was reduced in all stated amounts.

Table 1 shows the composition of starter and finisher diets and Table 2 presents the fatty acid content of lipids in the mixtures. A.O.A.C. (2002) methods were used for the analysis of basic chemical ingredients. Chickens were kept on the floor (straw). Feeding procedure was ad libitum. Control of temperature and relative humidity in the building showed that stated microclimatic factors were within the values optimal for Ross 208 broilers.

After the 42-day-long fattening period and 10-h-long hunger period, chickens were slaughtered. Samples of breast and thigh muscles were used for the purposes of this research. Skin and fatty tissue were carefully removed and muscles were chopped and homogenized. Composition of fatty acids in the lipids of white and red meat of broilers was determined on the 9 samples of each broiler group by the usage of Chrompack CP-9000 chromatograph with flame ionization detector. The determination procedure was as follows:

1.1. Digestion and fat extraction

Weigh the homogenized sample (which contains about 0.5–1 g fat) into an Erlenmeyer flask, add 8–20 ml concentrated hydrochloric acid and boil it on a steam-bath for 60–90 min. Let it cool down an add 7 ml of ethanol, then add 25 ml of diethyl ether, shake it vigorously for one minute. Add 25 ml of petrol ether (b.p. <60 °C) and shake it again for one minute. Wait until the two phases are separated, then pour about 20% of the organic phase (which contains about 150–200 mg fat) into a round-bottom flask and evaporate it under vacuum on a Rotadest. Complete evaporation is not required.

Table 1. Nutrient composition of the broiler diets

Ration ingredient (%)	Starter	Finisher				
		F_1	F_2	F_3	F_4	F_5
Corn	50.5	54.5	54.5	54.5	54.5	54.5
Soybean meal	31.8	30.3	30.3	30.3	30.3	30.3
Fish meal	8.0	5.0	5.0	5.0	5.0	5.0
Poultry fat	5.0	5.0	4.5	4.0	3.5	3.0
Limestone	2.0	2.0	2.0	2.0	2.0	2.0
Salt	0.2	0.3	0.3	0.3	0.3	0.3
Phosphonal	1.3	1.7	1.7	1.7	1.7	1.7
Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Premix[a]	1.0	1.0	1.0	1.0	1.0	1.0
PBE (marine oil)	0.0	0.0	0.5	1.0	1.5	2.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated contents						
Crude protein	22.67	20.43	20.43	20.43	20.43	20.43
Crude fat	7.89	7.86	7.86	7.82	7.80	7.78
Crude fibre	3.39	3.36	3.36	3.36	3.36	3.36
Lysine	1.41	1.22	1.22	1.22	1.22	1.22
Methionine	0.63	0.57	0.57	0.57	0.57	0.57
Methionine + cystine	0.97	0.90	0.90	0.90	0.90	0.90
Calcium	1.16	1.03	1.03	1.03	1.03	1.03
Total phosphorus	0.77	0.76	0.76	0.76	0.76	0.76
Sodium	0.19	0.19	0.19	0.19	0.19	0.19
ME MJ kg^{-1}	14.19	14.18	14.17	14.16	14.15	14.14

[a] Supplemented per kg of feed:
– vitamins: A 3.6 mg (12000 IU), D_3 0.05 mg (2000 IU), E 30 mg, K_3 2.5 mg, B_1 1.5 mg, B_2 6.0 mg, B_6 4.0 mg, B_{12} 0.015 mg, pantothenic acid 15 mg, nicotinic acid 20 mg, folic acid 0.5 mg, choline chloride 500 mg;
– microelements: Fe 30 mg, Cu 4.0 mg, Mn 80 mg, Zn 40 mg, Co 0.10 mg, Se 0.15 mg;
– other ingredients: antioxidant (butyl hydroxide toluol) 147 mg, lysine 1000 mg and methionine 500 mg

1.2. Hydrolysis and esterification

Add 4 ml of 0.5 M sodium-hydroxide in methanol, mount a cooler to the round-bottom flask and boil it on a water bath until the fat droplets disappear. Then add 4 ml of 14% boron-trifluoride in methanol through the cooler and boil it for 3 min. Add 2–6 ml of *n*-hexane and boil it for one minute then let it cool down. Bring the level of the organic phase to the neck of the flask with saturated sodium-chloride solution. When phases are separated, take samples for analyses from the organic phase, and dry it on sodium-sulphate.

Table 2. Fatty acid profile (% of total fatty acids) in diets

Fatty acid (% of total fatty acids)		Starter	Finisher				
			F_1	F_2	F_3	F_4	F_5
Capric	C 10:0	0.06	0.05	0.05	0.04	0.04	
Lauric	C 12:0		0.07	0.07	0.07	0.07	0.08
Tridecanoic	C 13:0	0.09	0.10	0.00	0.07	0.04	0.09
Myristic	C 14:0	1.39	1.44	1.94	2.17	2.73	3.16
Pentadecanoic	C 15:0	0.08	0.08	0.10	0.15	0.17	0.23
Palmitic	C 16:0	20.35	18.99	19.14	18.84	18.25	17.97
Heptadecanoic	C 17:0	0.31	0.31	0.38	0.39	0.39	0.45
Stearic	C 18:0	10.23	9.41	9.30	8.54	7.96	6.77
Arachidic	C 20:0	0.24	0.09	0.11	0.12	0.15	0.15
Heneicosanoic	C 21:0			0.11			
Behenic	C 22:0		0.09	0.08	0.08	0.10	0.10
Lignoceric	C 24:0		0.04		0.17	0.22	
Σ SFA		32.75	30.58	31.18	30.62	30.12	29.00
Palmitoleic	C 16:1	1.58	1.69	2.29	2.64	3.19	3.72
Oleic	C 18:1	32.11	30.60	30.04	29.29	26.76	24.53
Eicosenoic	C 20:1	0.61	0.67	0.76	0.91	0.87	1.05
Nervonic	C 24:1		0.08		0.04	0.04	
Erucic	C 22:1		0.04	0.11	0.29	0.25	0.53
Σ MUFA		34.30	33.08	33.20	33.17	31.12	29.00
Linoleic	C 18:2n6	23.37	24.39	21.56	20.59	19.89	20.08
Eicosadienoic	C 20:2n6	0.60	0.63	0.77	0.86	1.05	1.13
Eicosatrienoic	C 20:3n6		0.09	0.10		0.12	0.12
Arachidonic	C 20:4n6	0.22	0.26	0.34	0.39	0.52	0.57
Σ PUFA n-6		24.19	25.37	22.77	21.84	21.58	21.91
α-linolenic	C 18:3n3	1.28	1.31	1.36	1.27	1.23	1.26
Eicosapentaenoic	C 20:5n3	0.87	1.48	2.39	3.12	4.64	5.73
γ-docosapentaenoic	C 22:5n3	0.15	0.19	0.34	0.49	0.59	0.79
Docosahexaenoic	C 22:6n3	0.47	0.73	1.38	1.91	2.73	3.49
Σ PUFA n-3		2.77	3.71	5.47	6.78	9.18	11.26
Σ PUFA		26.96	29.08	28.24	28.62	30.76	33.17
SFA/MUFA		0.95	0.92	0.94	0.92	0.98	0.97
SFA/PUFA		1.21	1.05	1.10	1.07	0.98	0.87
PUFA n-6/PUFA n-3		8.73	6.84	4.16	3.22	2.35	1.94

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

1.3. Gas chromatography

Instrument: Chrompack CP 9000; column: 100 mm×0.25 mm wall coated open tubular (WCOT); stationary phase: CP-SIL 88 (FAME); detector: flame ionization detector (FID); injector: splitter; gases: carrier gas: helium, 235 kPa; at the detector: air:

250 ml min^{-1}, hydrogen: 30 ml min^{-1}, helium: 30 ml min^{-1}; temperatures injector: 270 °C; detector: 270 °C; column: 140 °C (10 min.), 10 °C min^{-1} increase up to 235 °C (26 min); injected volume: 0.5 µl.

Research results were evaluated by SAS statistic program Ver. 6.12.

2. Results and discussion

Fatty acid content in the lipids of breast muscles is shown in Table 3.

Table 3. Profile of fatty acids (% of total fatty acids) in breast muscles

Fatty acid		Group					*F* value
		1st (F_1) $\bar{x} \pm s$	2nd (F_2) $\bar{x} \pm s$	3rd (F_3) $\bar{x} \pm s$	4th (F_4) $\bar{x} \pm s$	5th (F_5) $\bar{x} \pm s$	
Lauric	C12:0	0.47±0.17	0.34±0.12	0.64±0.23	0.18±0.06	0.20±0.07	15.96**
Myristic	C14:0	0.82±0.12	1.22±0.17	1.27±0.30	1.51±0.43	1.50±0.32	8.36**
Pentadecanoic	C15:0	0.14±0.04	0.14±0.01	0.15±0.03	0.17±0.01	0.19±0.04	7.82**
Palmitic	C16:0	23.43±0.39	22.22±0.43	23.63±1.11	23.50±1.16	23.76±0.97	4.47**
Heptadecanoic	C17:0	0.30±0.04	0.34±0.05	0.33±0.01	0.35±0.02	0.38±0.03	8.90**
Stearic	C18:0	13.88±1.48	11.54±1.75	12.25±2.35	11.40±1.85	13.66±2.80	2.77*
Arachidic	C20:0	0.14±0.01	0.16±0.04	0.14±0.03	0.13±0.03	0.14±0.02	1.81
Behenic	C22:0	0.18±0.06	0.10±0.02	0.10±0.02	0.12±0.01	0.14±0.01	9.88**
Lignoceric	C24:0	0.12±0.04	0.12±0.05	0.12±0.05	0.14±0.02	0.17±0.02	3.78**
Σ SFA		39.48±1.63	36.18±1.95	38.63±2.97	37.50±2.40	40.15±3.37	3.46*
Palmitoleic	C16:1	1.52± 0.37	2.21±0.51	2.22±0.56	2.86±0.80	2.43±0.90	4.85**
Oleic	C18:1	26.02±2.37	29.15±2.60	25.52±3.13	26.58±1.87	23.54±3.17	5.16**
Eicosenoic	C20:1	0.34±0.05	0.48±0.09	0.42±0.07	0.49±0.05	0.59±0.10	13.77**
Nervonic	C24:1	1.73± 0.40	0.81±0.38	1.63±0.32	0.90±0.50	1.65±1.08	4.99**
Σ MUFA		29.61±2.37	32.65±2.71	29.79±3.52	30.83±2.38	28.21±2.85	3.09*
Linoleic	C18:2n6	13.38±1.19	14.72±1.84	12.94±2.26	12.89±2.24	11.14±1.77	16.82**
Eicosadienoic	C20:2n6	0.67± 0.05	0.63±0.03	0.68±0.05	0.69±0.03	0.63±0.07	3.01*
Arachidonic	C20:4n6	3.65±0.80	2.21±0.97	2.49±0.85	1.95±0.49	2.21±0.48	7.48**
Σ PUFA n-6		17.70±0.66	17.56±1.02	16.11+1.45	15.53±0.83	13.98±1.38	16.37**
α-linolenic	C18:3n3	0.52±0.10	0.82±0.18	0.61+0.20	0.75±0.09	0.66±0.10	5.98**
Eicosapentaenoic	C20:5n3	0.62±0.08	1.11±0.10	1.48±0.12	2.22±0.10	2.43±0.46	100.01**
Docosapentaenoic	C22:5n3	1.91±0.38	1.59±0.55	2.46±1.17	2.07±0.48	2.69±0.39	4.12**
Docosahexaenoic	C22:6n3	2.95±0.65	3.03±1.06	3.78±0.49	4.47±1.21	5.68±0.70	15.28**
Σ PUFA n-3		6.00±0.90	6.55±1.34	8.36±1.20	9.51±1.62	11.46±0.64	31.21**
SFA/MUFA		1.33	1.11	1.30	1.22	1.42	
SFA/PUFA		1.67	1.50	1.58	1.44	1.71	
PUFA n-6/PUFA n-3		2.95±0.48	2.68±0.71	1.93±0.44	1.63±0.41	1.22±0.40	21.39**

F 5% 2.61; F 1% 3.83; *P<0.5; **P<0.01.

Among saturated fatty acids in the lipids of breast muscles palmitic acid was the most dominant. Statistically significant differences ($P<0.01$) in the content of palmitic acid were observed in the lipids of breast muscles between the following groups: 1st and 2nd, 2nd and 3rd, 2nd and 4th, 2nd and 5th, 3rd and 4th, 3rd and 5th as well as between 4th and 5th group. Results related to the content of palmitic acid of experimental groups were in concordance with the results obtained by KRASICKA and co-workers (2000), who added soybean, linseed and sunflower oil into the forage mixtures. Based upon numerous researches (PINCHASOV & NIR, 1992; HRDINKA et al., 1996; KRALIK et al., 1997; 2001; CRESPO & ESTEVE-GARCIA, 2001), it is proven that chickens fed with diets with animal fats contained more saturated fatty acids, such as palmitic, stearic and myristic in the lipids of breast muscles, when compared with broilers which were fed with diets containing vegetable fats, especially linseed, soybean and rape oil.

Percentage of MUFA in the lipids of breast muscles changed from 28.21% in the 5th to 32.65% in the 2nd experimental group. Statistically significant differences ($P<0.05$) were established between the 1st and 2nd and between 2nd and 4th group, and highly significant differences ($P<0.01$) were observed between the 2nd and 3rd and 2nd and 5th group.

The lowest level of PUFA n-6 was established in the 5th experimental group, which was given food that contained 2% PBE preparation, and reached the value of 13.98%. Statistically highly significant differences ($P<0.01$) related to the total of n-6 fatty acids in the lipids of breast muscles were established between the 1st and 3rd, 1st and 4th, 1st and 5th, 2nd and 4th, 2nd and 5th, 3rd and 5th as well as between 4th and 5th group, and statistically significant differences ($P<0.05$) were observed between the 2nd and 3rd experimental group of broilers.

Compared to the control group, arachidonic acid of experimental groups was also decreased. This fatty acid, belonging to PUFA n-6 series, is not desirable for the human nutrition. Statistically highly significant differences ($P<0.01$) related to the decrease of arachidonic acid as a result of added PBE preparation in the mixtures were observed between control group and all experimental groups. Obtained results were in accordance with research results of LOPEZ-FERRER and co-workers (1999), who claimed that arachidonic acid was the product of elongation and desaturation of its linoleic acid precursor. The content of arachidonic fatty acid in this research was approximately the same as established by KOMPRDA and co-workers (1999), but lower than the values obtained by KRALIK and co-workers (2001).

Content of n-3 fatty acids in the breast muscles of the 1st control group was 6%. Addition of PBE preparation in the diets resulted in the increase of PUFA n-3 in the lipids of breast muscles. The highest concentration of n-3 fatty acids was found in the 5th experimental group, being 11.46%, which is 91% more when compared to the 1st group. In comparison to the 1st group, values of the 2nd, 3rd and 4th experimental groups were higher by 9.1%, 39% and 58%, respectively. Statistical data analysis showed highly significant differences ($P<0.01$) regarding concentration of PUFA n-3 in the lipids of breast muscles between the 1st and 3rd, 1st and 4th, 1st and 5th, 2nd and

4th, 2nd and 5th as well as between 3rd and 5th broiler groups, and statistically significant differences ($P<0.05$) were observed between the 2nd and 3rd and 4th and 5th group.

In comparison to the control group, content of eicosapentaenoic and docosahexaenoic acids was higher in all experimental groups. Our research results are in accordance with those obtained by HULAN and co-workers (1988), who found that the lipids of breast muscles of Arbor Acres broilers, slaughtered after the 42nd day of fattening contained 2.07% EPA and 5.25% DHA. Research results of KOMPRDA and co-workers (1999) and ZELENKA and co-workers (2001) showed less of these fatty acids for the same broiler hybrid. CRESPO and ESTEVE-GARCIA (2001) also discovered lower content of EPA and DHA in the lipids of breast muscles. Lower values obtained in the research can be explained by a limited and slow elongation and conversion of linoleic acid from the vegetable oils into EPA and DHA, as well as by the lower content of these fatty acids in the diets.

Changing of the content and PUFA n-6/PUFA n-3 ratio in the forage mixtures resulted in a better relation among these acids in the breast muscles. The lowest PUFA n-6/PUFA n-3 ratio, being 1.22, was established in the 5th experimental group, while the highest value, 2.95, was noticed in the 1st control group. Statistical analysis showed highly significant differences ($P<0.01$) in PUFA n-6/n-3 ratio between the 1st and 3rd, 1st and 4th, 1st and 5th, 2nd and 4th, 2nd and 5th and 3rd and 5th group of broilers, while statistically significant differences ($P<0.05$) existed between the 2nd and 3rd and between 4th and 5th experimental group. Average values of fatty acids in the lipids of red meat are shown in Table 4.

Statistical analysis confirmed highly significant differences ($P<0.01$) in the content of saturated fatty acids between the 1st and 2nd group, 1st and 4th, 2nd and 4th, 2nd and 5th, as well as between 3rd and 4th and 3rd and 5th group. Statistically significant differences ($P<0.05$) were found between the 1st and 5th group. The sum of saturated fatty acids in the red meat was considerably higher in our research than in those done by ZELENKA and co-workers (2001), who used commercial feedstuffs and mixtures with limited portion of maize and wheat. Compared to our research, lower content of saturated fatty acids in the lipids of thighs was established in researches of CRESPO and ESTEVE-GARCIA (2001). Furthermore, when comparing the sum of saturated fatty acids in the lipids of breast muscles with the sum of the same fatty acids in the lipids of thighs, we noticed greater concentration of unsaturated fatty acids in the breast muscles. When comparing the sum of MUFA in the lipids of breast muscles with the sum of the same fatty acids in the lipids of thighs, a greater concentration of MUFA was observed in thigh lipids. Almost identical results of concentrated fatty acids were obtained by KRASICKA and co-workers (2000). In the experiments of those authors chickens were fed with diets enriched with full-fat linseed, rapeseed and soybean oil.

Table 4. Profile of fatty acids (% of total fatty acids) in the thigh muscles

Fatty acid		Group					*F* value
		1st (F_1) $\bar{x} \pm s$	2nd (F_2) $\bar{x} \pm s$	3rd (F_3) $\bar{x} \pm s$	4th (F_4) $\bar{x} \pm s$	5th (F_5) $\bar{x} \pm s$	
Lauric	C12:0	0.31±0.13	0.15±0.06	0.13±0.06	0.16±0.07	0.25±0.17	4.33**
Myristic	C14:0	1.05±0.07	1.38±0.08	1.62±0.10	1.67±0.19	1.87±0.12	59.44**
Pentadecanoic	C15:0	0.13±0.05	0.13±0.03	0.15±0.01	0.25±0.13	0.17±0.04	4.79**
Palmitic	C16:0	21.79±0.41	20.51±0.28	21.60±0.34	22.62±1.49	22.30±0.91	8.62**
Heptadecanoic	C17:0	0.30±0.03	0.36±0.03	0.37±0.03	0.38±0.04	0.39±0.01	12.57**
Stearic	C18:0	9.90±1.06	8.11±1.08	8.33±0.75	10.98±2.63	10.47±1.45	6.23**
Arachidic	C20:0	0.16±0.04	0.14±0.03	0.12±0.02	0.14±0.01	0.13±0.02	3.06*
Behenic	C22:0	0.10±0.03	0.07±0.02	0.06±0.04	0.12±0.01	0.13±0.01	16.23**
Lignoceric	C24:0	0.09±0.01	0.09±0.01	0.10±0.02	0.17±0.02	0.17±0.02	81.27**
Σ SFA		33.83±1.31	30.94±1.20	32.48±0.96	36.49±1.98	35.88±1.82	10.37**
Palmitoleic	C16:1	2.72±0.42	3.44±0.37	3.75±0.28	3.16±0.79	3.76±0.51	6.84**
Oleic	C18:1	31.54±1.62	31.99±1.39	32.01±1.40	25.96±3.09	24.45±3.79	20.00**
Eicosenoic	C20:1	0.47±0.03	0.56±0.03	0.61±0.06	0.50±0.13	0.62±0.10	6.30**
Nervonic	C24:1	0.46±0.19	0.23±0.14	0.33±0.17	0.61±0.31	0.62±0.35	4.81**
Σ MUFA		35.63±1.92	36.22±1.55	36.70±1.51	30.23±3.68	29.45±3.77	14.83**
Linoleic	C18:2n6	17.23±0.64	17.77±0.98	16.52±0.36	15.27±0.66	13.90±1.12	34.25**
Eicosadienoic	C20:2n6	0.52±0.04	0.55±0.03	0.57±0.01	0.64±0.04	0.62±0.07	11.53**
Arachidonic	C20:4n6	2.10±0.63	1.46±0.41	1.17±0.29	2.02±0.66	2.15±1.01	4.16**
Σ PUFA n-6		19.85±0.38	19.78±0.81	18.26±0.57	17.93±0.50	16.67±0.48	50.30**
α-linolenic	C18:3n3	0.82±0.08	0.98±0.09	0.91±0.09	0.74±0.17	0.70±0.22	6.85**
Eicosapentaenoic	C20:5n3	0.48±0.05	1.18±0.23	1.30±0.35	2.24±0.12	2.76±0.36	112.49**
Docosapentaenoic	C22:5n3	0.96±0.26	0.89±0.18	1.13±0.21	1.81±0.28	2.07±0.52	25.81**
Docosahexaenoic	C22:6n3	1.45±0.42	1.83±0.53	2.83±0.51	3.76±0.95	4.91±0.95	16.49**
Σ PUFA n3		3.71±0.59	4.88±0.76	6.17±1.01	8.55±1.06	10.44±1.26	21.77**
SFA/MUFA		0.95	0.85	0.89	1.21	1.22	
SFA/PUFA		1.44	1.26	1.33	1.38	1.32	
PUFA n-6/PUFA n-3		5.35±0.81	4.05±0.68	2.96±0.47	2.10±0.28	1.60±0.37	43.19**

F 5% 2.61; F 1% 3.83; **P<0.01.

Among monounsaturated fatty acids in the lipids of thighs, the most represented one was oleic acid. Statistically highly significant differences (P<0.01) of oleic acid in the lipids of thighs were observed between the 1st and 4th, 1st and 5th, 2nd and 4th, 2nd and 5th, 3rd and 4th, and between the 3rd and 5th group.

Values of oleic acid in the thighs were in our research lower than those established by KRASICKA and co-workers (2000) and by CRESPO and ESTEVE-GARCIA (2001).

The sum of PUFA n-6 in the lipids of thighs fluctuated from 19.85% in the 1st group to 16.67% in the 5th group. Addition of PBE preparation resulted in the decrease of all n-6 fatty acids in food, except for the 5th group, and consequently also in the lipids of thighs. With statistical analysis of all n-6 fatty acids in the lipids of thighs, highly significant differences ($P<0.01$) among all examined groups were found, except for the 1st control group and the 2nd group, as well as 3rd and 4th experimental group.

Comparing the obtained values of total PUFA n-6 in the lipids of breast muscles with PUFA n-6 in thighs, we observed greater tendency of PUFA n-6 deposition in the lipids of thighs. The work of KRASICKA and co-workers (2000) showed identical results.

Feeding broilers with diets containing PBE preparation resulted in the increase of total n-3 fatty acids in the lipids of thigh muscles. Sum of PUFA n-3 in our experiments varied from 3.71% in the 1st group to 10.44% in the 5th group. Highly significant differences ($P<0.01$) regarding the total n-3 fatty acids in the lipids of thighs were noticed between all groups with higher portion of n-3 fatty acids, except between 1st (control) and 2nd group.

From statistical point of view, research works showed that food enriched with PBE preparation in the amount of 0.5%, 1%, 1.5% and 2% affected the increase of EPA and DHA in the lipids of thigh muscles in all experimental groups. The highest content of unsaturated n-3 fatty acids was established in the lipids of thighs of the 5th group, which was given diets containing 2% PBE preparation. For that reason the content of EPA and DHA in the lipids of thigh muscles was increased from 0.48% and 1.45% (1st group) to 1.18% and 1.83% (2nd group), 1.30% and 2.83% (3rd group), 2.24% and 3.76% (4th group) and 2.76% and 4.91% (5th group).

Results of our experiments related to the EPA and DHA in the lipids of thighs were higher than the results of those conducted by CRESPO and ESTEVE-GARCIA (2001). In this research the content of EPA varied from 0.02% to 0.9%, and the content of DHA from 0.12% to 0.63%, depending upon the source of fat in the forage mixtures.

PUFA n-6/n-3 ratio in the lipids of thigh muscles was decreasing gradually by adding of PBE preparation. For that reason, ratio between the stated fatty acids in the thigh muscles was 5.35 in the 1st group, while the ratio of the 5th group, which consumed the most PBE preparation, was considerably lowered to 1.60. Results of PUFA n-6/n-3 ratio obtained throughout our research were more agreeable than the ratio stated by ZELENKA and co-workers (2001) and CRESPO and ESTEVE-GARCIA (2001).

3. Conclusion

Research results related to the possibility of PUFA n-3 increase in the broiler meat by addition of Pronova Biocare Epax 3000 TG (marine oil enriched by EPA and DHA) in the amount of 0%, 0.5%, 1%, 1.5% and 2% as a replacement for poultry fat, showed that the content of PUFA n-3 in the lipids of mixtures affected their greater deposition in the lipids of muscular tissue, and at the same time lowered the content of PUFA n-6. Adding certain amounts of PBE preparation in the diets resulted in PUFA n-3 amounts of 6%, 6.55%, 8.36%, 9.51% and 11.46% in the lipids of breast muscles, while these amounts in the lipids of thigh muscles were the following: 3.71%, 4.88%, 6.17%, 8.55% and 10.44%. Consequently, PUFA n-6/n-3 ratio was lowered from 2.95 to 1.22 in the breast muscles, and from 5.35 to 1.60 in the thigh muscles.

References

ALBRECHT, M. & KLEIN, M. (1995): Oleum Lini: Portrait eines pflanzlichen Oels. *Pharmazie*, *7*, 36–40.

A.O.A.C. (2002): *Official Methods of Analysis*, A.O.A.C. International, 17th Edition, Revision 1, Volume I, Chapter 4 (Animal Feed), pp. 1–45.

BARLOW, S. & PIKE, I.M. (1991): Humans, animals benefit from omega-3-polyunsaturated fatty acids. *Feedstuffs*, *63*, 18–26.

CHANMUGAM, P., BOUDREAU, M., BOUTTE, T., PARK, R.S., HEBERT, J., BERRIO, L. & HWANG, D.W. (1992): Incorporation of different types of n-3 fatty acids into tissue lipids of poultry. *Poultry Sci.*, *71*, 516–521.

CRESPO, N. & ESTEVE-GARCIA, E. (2001): Dietary fatty acid profile modifies fat deposition in broiler chickens. *Poultry Sci.*, *80*, 71–78.

HRDINKA, C., ZOLLITSCH, W., KNAUS, W. & LETTNER, F. (1996): Effects of dietary fatty acid pattern on melting point and composition of adipose tissues and intramuscular fat of broiler carcasses. *Poultry Sci.*, *75*, 208–215.

HULAN, H.W., PROUDFOOT, F.G. & NASH, D.M. (1984): The effect of different diet fat sources on general performance and carcass fatty acid composition of broiler chickens. *Poultry Sci.*, *63*, 324–332.

HULAN, W.H., ACKMAN, R.G., RATNAYAKE, W.M.N. & PROUDFOOT, G.F. (1988): Omega-3 fatty acid levels and performance of broiler chickens fed redfish meal or redfish oil. *Can. J. Anim. Sci.*, *68*, 533–547.

KOMPRDA, T., ZELENKA, J., TIEFFOVA, P., ŠTOHANDLOVA, M. & FOLTYN, J. (1999): Effect of the composition of commercial feed mixture on total lipid, cholesterol and fatty acids content in broiler chicken meat. *Czech. J. Anim. Sci.*, *44*, 179–185.

KRALIK, G., BOŽIČKOVIĆ, P., GALONJA, M., ŠKRTIĆ, Z. & CANECKI, K. (1997): Mogućnost povećanja sadržaja višestruko nezasićenih masnih kiselina u pilećem mesu putem hranidbe. (The possibility of increasing the content of polyunsaturated fatty acids in broiler meat by feeding.) *Krmiva*, *39*, 223–231.

KRALIK, G., KUŠEC, G. & IVANKOVIĆ, S. (2001): Effect of dietary oils on fatty acids content in chicken meat. *Congress Proceedings, 47th International Congress of Meat Science and Technology (ICoMST)*. Kraków, Poland, 26–31 Aug., Vol. I, 3–P28, pp. 230–231.

KRALIK, G., IVANKOVIĆ, S. & ŠKRTIĆ, Z. (2002): Mijenjanje profila masnih kiselina u mišićnom tkivu brojlera. (Changing the profile of fatty acids in muscle tissue of broilers.) *Krmiva*, *44*, 297–305.

KRASICKA, B., KULASEK, G.W., SWIERCZEWSKA, E. & ORZECHOWSKI, A. (2000): Body gains and fatty acid composition in carcasses of broilers fed diets enriched with full-fat rapeseed and/or flaxseed. *Arch. Geflügelkunde*, *64*, 61–69.

LEAF, A. & WEBER, P.C. (1988): Cardiovascular effects of ω3-fatty acids. *N. Engl. J. Med.*, *318*, 549.

LOPEZ-FERRER, S., BAUCELLS, M.D., BARROETA, A.C. & GRASHORN, M.A. (1999): Influence of vegetable oil sources on quality parameters of broiler meat. *Arch. Geflügelkunde*, *63*, 29–35.

OKUYAMA, H. & IKEMOTO, A. (1999): Needs to modify the fatty acid composition of meats for human health. *Proceedings of 45 ICoMST*. Yokohama, Japan, pp. 638–640.

PINCHASOV, Y. & NIR, I. (1992): Effect of dietary polyunsaturated fatty acid concentration on performance, fat deposition, and carcass fatty acid composition in broiler chickens. *Poultry Sci.*, *71*, 1504–1512.

SANZ, M., FLORES, A., PEREZ DE AYALA, P. & LOPEZ-BOTE, C.J. (1999): Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *Brit. Poultry Sci.*, *40*, 95–101.

SCAIFE, J.R., MOYO, J., GALBRAITH, H., MICHIE, W. & CAMPBELL, V. (1994): Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. *Brit. Poultry Sci.*, *35*, 107–118.

ZELENKA, J., KOMPRDA, T. & FAJMONOVA, E. (2001): Mastne kyseliny v mase restringovane vykrmenych kurat. (Fatty acids in meat of restrictively fattened chickens.) *IV. Kabrtovy dieteticke dny, Konference s mezinarodni účasti Veterinarni a Farmaceuticka Univerzita Brno*, pp. 22–25.

ZOLLITSCH, W., KNAUS, W., AICHINGER, F. & LETTNER, F. (1997): Effects of different fat sources on performance and carcass characteristics of broilers. *Anim. Feed Sci. Technol.*, *66*, 63–73.