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Arachidonic Acid and Long-Chain n−3 Polyunsaturated Fatty Acid Contents in Meat of Selected Poultry and Fish Species in Relation to Dietary Fat Sources

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Arachidonic acid (AA) content, long-chain n−3 polyunsaturated fatty acid (PUFA) equivalent [LCE; calculated as 0.15 × linolenic acid (LA) + eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)], and PUFA n−6/PUFA n−3 ratio were determined in meat [breast meat (BM), thigh meat (TM), and fillets (F), respectively] within four sets of chickens, five sets of turkeys, one set of common carp, and four sets of rainbow trout, fed either commercial diet or diets with manipulated PUFA n−3 and PUFA n−6 contents. AA content was within the range of 20 mg/100 g (F of rainbow trout fed the diet with linseed oil, LO) to 138 mg/100 g (TM of chickens fed restrictively the diet based on maize to the age of 90 days). AA content in BM of turkeys fed the diet with LO or fish oil (FO) did not differ ($P > 0.05$) from that of rainbow trout F. LCE was in the range of 16 mg/100 g (BM of turkeys fed a commercial feed mixture) to 681 mg/100 g (F of rainbow trout fed a commercial feed mixture). With regard to BM, only turkeys fed the diet with LO deposited more ($P < 0.01$) LCE (71 mg/100 g) as compared to all other poultry sets except turkeys fed the diet with FO (123 mg/100 g). Apart from all fish samples, also both BM and TM of turkeys fed the diet with either LO or FO met the recommended value of the PUFA n−6/PUFA n−3 ratio (<4). AA content in the tissue increased significantly ($P < 0.001$) with increasing dietary LA in both all chicken tissues and all turkey tissues, which is contrary to the suggested strong metabolic regulation of the AA formation. When all tissues within all animal species were taken as a one set, both AA percentage and EPA + DHA percentage in the tissue (Y, %) decreased ($P < 0.001$) with increasing fat content in the tissue (X, %), according to the equation $Y = 4.7 - 0.54X$ ($R^2 = 0.41$) and $Y = 6.0 - 0.33X$ ($R^2 = 0.35$), respectively. AA content in chicken BM, chicken TM, and turkey BM, respectively, decreased linearly ($P < 0.01$) with increasing live weight reached at the slaughter age.

KEYWORDS: Arachidonic acid; EPA; DHA; healthy nutrition; chicken; turkey; rainbow trout

INTRODUCTION

The importance of a relatively high intake of polyunsaturated fatty acids (PUFA) in human nutrition is nowadays generally accepted; PUFA should constitute 7% of total energy consumed (*1*). Within PUFA, fatty acids essential for man (they must be consumed in food) are linoleic acid (C18:2n−6; LA) and α-linolenic acid (C18:3n−3; LNA), the precursors of PUFA n−6 and n−3 series, respectively. The quantitatively and qualitatively most important metabolites of LA and LNA are arachidonic acid (C20:4n−6; AA) and eicosapentaenoic acid (C20:5n−3; EPA) and docosahexaenoic acid (C22:6n−3; DHA), respectively. DHA and AA are the major PUFA in the membranes of brain and retinal cells and have an impact on neuronal functions (*2*).

Eicosanoids (prostaglandins, thromboxanes, and leucotrienes) derived from AA, on the one hand, and from EPA and DHA, on the other hand, have different physiological effects on man (*3*). Proinflammatory and proaggregatory AA derived eicosanoids increase the risk of cardiovascular (*4*) and autoimmune diseases (*5*). On the other hand, anti-inflammatory, antithrombotic, antiarrhythmic, and immunomodulating properties of EPA and DHA can be helpful in the prevention of atherosclerosis (*6*), coronary heart diseases (*7*, *8*), hypertension, inflammatory (*9*) and autoimmune disorders (*10*), cancers (*11*), and diabetes (*12*).

From the above-mentioned follows the demand to keep the proper n−6/n−3 PUFA ratio in the diet (*13*). Epidemiological data suggest (*14*) that increasing n−6/n−3 PUFA ratio led to the rapid increase in mortalities from Western-type cancers and allergies in Japan. Therefore, Okuyama et al. (*14*) recommended the n−6/n−3 PUFA ratio to be ≤2.

With regard to inflammatory diseases, AA intake should be <90 mg/day (*15*); Taber et al. (*16*) estimated an average AA

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Table 1. Sets of Experimental Animals, Diet Characterization, and Methods of Fattening

set	animal species	gender	number	age at slaughter (days)	live weight at slaughter (g)	diet	method of fattening
1[a]	chicken[c]	male	48	43	1100–2500[j]	commercial	ad libitum
2[b]	chicken[c]	female	12	87	2200	wheat/maize meal 2/1[j]	restrictive[p]
3[b]	chicken[c]	female	12	90	2200	wheat/maize meal 1/2[k]	restrictive[p]
4[b]	chicken[c]	female	12	74	2200	wheat/maize meal 1/2[k]	semi ad libitum[q]
5	turkey[d]	male	53	140	14000–24000[j]	commercial	ad libitum
6	turkey[d]	ND[g]	14	56	3370	commercial	ad libitum
7	turkey[d]	ND[g]	14	56	3370	commercial + LO[l]	ad libitum
8	turkey[d]	ND[g]	14	56	3370	commercial + SO[m]	ad libitum
9	turkey[d]	ND[g]	14	56	3370	commercial + FO[n]	ad libitum
10	common carp[e]	male + female[h]	48	1100	1470–2560[j]	wheat	ad libitum
11	rainbow trout[f]	ND[g]	16	440	422	commercial	ad libitum
12	rainbow trout[f]	ND[g]	16	440	423	commercial + LO[l]	ad libitum
13	rainbow trout[f]	ND[g]	16	440	423	commercial + SO[m]	ad libitum
14	rainbow trout[f]	ND[g]	16	440	422	commercial + LO + SO[o]	ad libitum

[a] Cockerels were taken in four different cycles of commercial fattening (10, 12, 14, and 12 birds, respectively); [b] Chickens were fed (from the age of 6 weeks until the end of the experiment, that is, when the birds reached a live weight of 2200 g) intentionally slowly the exclusively cereal diets. [c] Ross 208. [d] BUT Big 6. [e] *Cyprinus carpio.* [f] *Oncorhynchus mykiss.* [g] Sex was not distinguished. [h] In equal parts. [i] Animals were taken in as broad a range of live weight as possible to evaluate (apart from the mutual comparison with the other sets) an effect of live weight at slaughter on fatty acid content in the tissue. [j] Exclusive cereal mixture (without soybean meal), two parts of wheat and one part of maize meal. [k] Exclusive cereal mixture (without soybean meal), one part of wheat and two parts of maize meal. [l] Linseed oil was added to the commercial feed mixture in the amount of 5% of fresh matter. [m] Sunflower oil was added to the commercial feed mixture in the amount of 5% of fresh matter. [n] Fish oil was added to the commercial feed mixture in the amount of 5% of fresh matter. [o] Linseed oil and sunflower oil were added to the commercial feed mixture in the amounts of 2.5 and 2.5% of fresh matter, respectively. [p] Qualitative and quantitative restriction (low crude protein content, energy/protein ratio 94.2 kJ AME_n/g of crude protein, 85–60% of the ad libitum intake between the 43rd and 90th days of fattening). [q] Offered only in such amount of feed that the birds were able to ingest without refusals (~95% of the ad libitum intake).

intake to be 100−500 mg/day. Fat from poultry meat could be a significant contribution to the dietary intake of AA (*17*). Information on how much AA is actually consumed is sometimes conflicting (*16*); according to Mann et al. (*18*), actual AA intake is lower than sometimes estimated.

Increasing the consumption of fish (especially fatty fish) can decrease the n−6/n−3 PUFA ratio, which is currently 10 or more in many population groups with the so-called Western-type of consumption. However, it has been estimated that >85% of the world fish oil supplies will be used for aquaculture production by the year 2010, and therefore the use of plant oils (especially linseed oil, rich in LNA) as a component of the fish diets was suggested (*19*).

On the other hand, due to the current limited availability and high cost of fish and the low acceptance of fish meat to many consumers, meat, dairy products, and eggs enriched by n−3 PUFA seem to be a feasible alternative. On the basis of the results of recent experiments, feeding of linseed oil to pigs increased LNA and long-chain n−3 PUFA in muscle and backfat at the expense of AA (*20*). The addition of flaxseed oil to the diets fed to hens allowed the production of eggs with higher EPA and DHA content and lower n−6/n−3 PUFA ratio (*21*). Transfer of dietary EPA and DHA to milk fat is very low due to the extensive biohydrogenation in the rumen. Strategies to enhance the nutritive value of milk fat therefore involve increasing rumen outflow of vaccenic acid (rumen biohydrogenation intermediate from both LA and LNA) and increasing Δ9-desaturase activity in the mammary gland to increase milk fat *cis*-9,*trans*-11 conjugated linoleic acid isomer with anticarcinogenic and antiatherogenic activities found in animal models (*22*).

Fish oil (*23*) or linseed oil and rapeseed oil (*24*) are used most commonly in the diets with an aim to manipulate the n−3 PUFA composition of poultry meat. Similarly, the fatty acid profile of ostrich meat was altered as a result of the consumption of fish oil (*25*). Schreiner et al. (*26*) used seal blubber oil as an alternative to fish oil to manipulate the n−3 fatty acid profile in broiler meat. Both EPA and DHA concentrations in poultry meat can be increased when the diet is supplemented with these fatty acids, but supplementing the diet with LNA does not result in a noticeable increase in EPA content in poultry meat (*27*).

The objectives of the present study were as follows. The first purpose was to compare AA content, long-chain PUFA content, and PUFA n−6/PUFA n−3 ratio in different tissues within two poultry species and two freshwater fish species (commonly consumed in Europe). The above values were determined in the series of experiments carried out at the comparable conditions in altogether 14 sets of animals fed the controlled diets (commercial feed mixtures and the diets with manipulated PUFA n−6 and PUFA n−3 contents) and can therefore be used within the current effort to validate and update the databases for fatty acid composition of foods (*16*). Second, because altogether 23 tissues within four animal species were evaluated, some generalized relationships between AA content, EPA + DHA content, and PUFA n−6/PUFA n−3 ratio in the tissue, on the one hand, and dietary LA, dietary LNA, and LA/LNA ratio in the diet, respectively, fat content in the tissue, and live weight reached at slaughter age (i.e., growth intensity), on the other hand, could be assessed.

MATERIALS AND METHODS

Animals and Diets. Fourteen sets of experimental animals (including the diets and the methods of fattening) are characterized in **Table 1**. Percentages of the quantitatively most important fatty acids in particular finishing feed mixtures, including total lipid content, are presented in **Table 2**.

All of the conditions, at which all experimental animals were kept, including stocking density, were in full agreement with the current European Union directives regarding animal welfare.

Sample Preparation. Immediately after slaughter, the following tissues were separated: in chickens, breast muscles without skin and external visible fat (breast meat, BM), and thigh muscles without skin and external visible fat (thigh meat, TM); in turkeys, musculus pectoralis profundus (breast meat, BM), and m. biceps femoris + m. semitendinosus + m. semimembranosus (thigh meat, TM), both parts rid of skin and visible external fat; fish were filleted. The separated tissues were homogenized in a Moulinex blender (model D56, Moulinex, France), put into the dark glass powder bottles, frozen, and stored at −20 °C until fatty acid analyses.

Extraction of Total Lipid from Animal Tissues. The particular tissue was thawed at the room temperature and weighed into the 500

Table 2. Fat Content and Percentage of Physiologically Important Fatty Acids in the Finishing Diets Used in the Experiment

		fatty acid (% of total determined fatty acids)					
set[a]	fat[b] (g/100 g)	C16:0	C18:1n–9	C18:2n–6	C18:3n–3	EPA + DHA[c]	n–6/n–3 ratio
1	49	13.4	37.0	37.6	4.1	0.3	8.5
2	26	24.1	18.8	43.7	2.5	0.1	16.8
3,4	29	18.8	23.9	46.7	1.8	0.1	24.6
5	41	18.2	31.6	38.6	1.9	0.5	16.2
6	36	17.6	32.3	37.5	1.9	1.7	10.4
7	84	10.2	20.7	25.3	36.5	1.1	0.7
8	85	10.6	26.1	54.7	1.1	1.2	23.8
9	80	19.2	30.0	19.6	1.3	7.7	2.2
10	50	17.9	15.8	22.1	11.5	3.1	1.5
11	146	17.0	19.7	20.8	3.2	18.7	0.9
12	189	13.8	17.9	18.0	20.6	13.7	0.5
13	184	13.7	21.9	32.1	2.3	13.5	2.0
14	182	13.7	19.8	24.8	11.4	13.9	1.0

[a] 1, commercial feed mixture, fed for 28 days before slaughter (chickens); 2, cereal feed mixture based on wheat (two parts of whole wheat, one part of maize meal, fed for 44 days before slaughter, chickens); 3, 4, cereal feed mixture based on maize (two parts of maize meal, one part of wheat, fed for 44 days before slaughter, chickens); 5, commercial feed mixture, fed for 56 days before slaughter (turkeys); 6, commercial feed mixture, fed for 35 days before slaughter (turkeys); 7, same feed mixture as in set 6 with linseed oil added in the amount of 5% of fresh matter, fed for 35 days before slaughter (turkeys); 8, same feed mixture as in set 6 with sunflower oil added in the amount of 5% of fresh matter, fed for 35 days before slaughter (turkeys); 9, same feed mixture as in set 6 with fish oil added in the amount of 5% of fresh matter, fed for 35 days before slaughter (turkeys); 10, a diet based on wheat, fed for 330 days before slaughter (carp); 11, commercial feed mixture, fed for 70 days before slaughter (rainbow trout); 12, same feed mixture as in set 11 with linseed oil added in the amount of 5% of fresh matter, fed for 70 days before slaughter (rainbow trout); 13, same feed mixture as in set 11 with sunflower oil added in the amount of 5% of fresh matter, fed for 70 days before slaughter (rainbow trout); 14, same feed mixture as in set 11 with linseed oil and sunflower oil added in the amounts of 2.5 and 2.5% of fresh matter, respectively, fed for 70 days before slaughter (rainbow trout). [b] Determined as diethyl ether extract after 12 h of extraction under reflux. [c] Eicosapentaenoic acid (C20:5n–3) + docosahexaenoic acid (C22:6n–3).

mL Erlenmeyer flask according to the presumed total lipid content: 70 g of the rainbow trout fillet and poultry breast meat, respectively, and 50 g of carp fillet and chicken and turkey thigh meat, respectively. The muscle tissue was spiked with 5 mL of the internal standard solution for fatty acid determination: 2.5 mg of C15:0/mL of isooctane (Supelco).

The sample was then extracted for 1 min with 180 mL of hexane/2-propanol (HIP) 3:2 v/v mixture (HIP 1) using the DIAX 900 homogenizer (Heidolph, Germany). The mixture was filtered through the Büchner funnel. One hundred and twenty milliliters of the aqueous solution of Na_2SO_4 (1 g of anhydrous salt per 15 mL of water) was added. After shaking and separation of the layers in the separation funnel, the *n*-hexane layer was filtered through anhydrous Na_2SO_4 to a 250 mL volumetric flask. The water layer was re-extracted with 50 mL of HIP (7:2, v/v; HIP 2).

The *n*-hexane layer after re-extraction and drying with anhydrous Na_2SO_4 was added to the 250 mL volumetric flask and filled to the mark with *n*-hexane. Combined extracts were transferred to the 500 mL round-bottom flask, and the content was evaporated on a rotary vacuum evaporator (RV 05-ST 1P-B model; IKA Labortechnik, Germany) at 40 °C. Evaporation was finished under nitrogen, and total lipids were determined gravimetrically.

Fatty Acid Determination. An aliquot of 40−60 mg of the cleaned HIP extract was evaporated to dryness under nitrogen, the solid residue was weighed in the reaction flask, and 1 mL of butylated hydroxytoluene (BHT; 1% solution in CH_3OH; Sigma) to prevent oxidation was added. Two milliliters of a 0.5 N methanolic solution of CH_3ONa (1.15 g of Na/100 mL of CH_3OH) was added after ultrasonic homogenization, and the mixture was boiled for 5 min under reflux. Two milliliters of a 14% solution of BF_3 in CH_3OH was added through the condenser, and the mixture was refluxed for another 5 min. Heating was discontinued, 2 mL of isooctane was added, and the sample was shaken and left to stand for 1 min. Five milliliters of a saturated aqueous solution of NaCl was added, and the mixture was shaken vigorously for 15 s while tepid. The organic layer was transferred into the test tube, and 2 μL was injected into the gas−liquid chromatography (GLC) column.

FA methyl esters (FAMEs) were separated using an HP 4890 chromatograph (Hewlett-Packard) and a capillary column Omegawax TM250 30 m × 0.25 mm × 0.25 μm with the following temperature program: 205/240 °C, held for 9/16 min. The rate of heating was 5 °C/min; the injector temperature was 280 °C and the detector temperature 300 °C. Carrier gas was N_2 at a flow rate of 0.9 mL/min. PUFA no. 2 (Animal Source, Supelco) and Oil Reference Standard no. 6 (Sigma) were used as external standard in FAMEs identification in addition to the internal standard. The equivalent of long-chain n−3 PUFA (LCE; in mg/100 g of the tissue) was calculated from the determined content of LNA, EPA, and DHA according to the method given in ref *28*: LCE = 0.15 LNA + EPA + DHA (assuming that only 15% of LNA can be converted to EPA and DHA rapidly enough to satisfy the needs of an organism).

Due to the several steps of the sample preparation, the concentration of fatty acid in the sample (in mg/100 g) was corrected on the basis of the method of internal standard. Because the absolute amount of each FA in the tissue depends on the lipid content in the particular tissue, the FA percentage in the sum of total determined fatty acids was also calculated, apart from expressing fatty acid content in milligrams per 100 g of the fresh sample.

Statistical Evaluation. Comparison of total lipid content and particular fatty acid content, respectively, in the animal tissues was based on the one-way analysis of the variance ratio test, including Duncan's multiple-range test, at the significance level of $P < 0.01$. The relationships between AA percentage, EPA + DHA percentage, and PUFA n−6/PUFA n−3 ratio in the tissue, on the one hand, and dietary LA, dietary LNA, and LA/LNA ratio in the diet, respectively, fat content in the tissue, and live weight reached at slaughter age, on the other hand, were evaluated using a regression analysis; the significance of the linear term and quadratic term, respectively, was tested. The Unistat package, version 4.53 (Unistat Ltd., London, U.K.), was used for the above tests, including the calculation of the basic statistical characteristics.

RESULTS AND DISCUSSION

Comparison of Tissues. Total lipid content (HIP extract; **Table 3**) in the evaluated tissues was in the range of 0.7 (BM of turkeys fed a commercial diet) to 8.8 g/100 g (carp fillet). The mean total lipid content (all corresponding tissues irrespective of dietary fat source were taken as a one set) increased ($P < 0.05$) in the sequence turkey BM (0.9) = chicken BM (1.5) < turkey TM (2.6) < rainbow trout fillet (3.8) < chicken TM (5.1) < carp fillet (8.0 g/100 g). Lipid content in rainbow trout fillet is lower in comparison with literature data [8.4−14.9% (*29*)]. This could be explained by the fact that, despite feeding ad libitum, the dosage was relatively low due to the low water temperature (8 °C), as it corresponded to the recommendation of the producer of the feed mixture.

With regard to food composition databases, it is necessary to know absolute values of fatty acids content in the products. Therefore, arachidonic acid content (**Figures 1** and **4**) and long-chain PUFA content (**Figure 2**) are expressed this way (mg of FA/100 g of product) in the present experiment, in accordance with a substantial part of the pertinent literature data (*16*, *17*, *30*). On the other hand, some authors present FA content as a percentage in the sum of total determined fatty acids (*23*, *29*), because the absolute amount of each FA in the tissue depends not only on the FA composition of the diet but also on the lipid content of the diet and, consequently, on the lipid content in the particular tissue. To be able to compare the results, AA

Table 3. Fat Content and Percentage of Nutritionally Important Fatty Acids in Chicken, Turkey, Common Carp, and Rainbow Trout Meat (Mean[a] ± Standard Error of the Mean)

set[b]	*n*	HIP extract[c]	C16:0[d]	C18:1n–9[d]	C18:2n–6[d]	C18:3n–3[d]	C20:4n–6[d]	EPA + DHA[d,e]
1a	49	$1.7^{B} \pm 0.1$	$22.8^{E} \pm 0.2$	$38.7^{KL} \pm 0.4$	$18.6^{D} \pm 0.3$	$1.4^{BC} \pm 0.1$	$2.2^{BC} \pm 0.1$	$1.2^{BC} \pm 0.1$
1b	49	$7.2^{H} \pm 0.2$	$22.8^{E} \pm 0.2$	$41.2^{M} \pm 0.3$	$18.5^{D} \pm 0.4$	$1.6^{C} \pm 0.1$	$0.9^{A} \pm 0.0$	$0.4^{A} \pm 0.0$
2a	12	$1.3^{AB} \pm 0.0$	$24.2^{FG} \pm 0.2$	$37.1^{K} \pm 0.4$	$11.3^{B} \pm 0.2$	$0.4^{A} \pm 0.0$	$6.5^{H} \pm 0.2$	$1.3^{BCD} \pm 0.0$
3a	12	$1.6^{AB} \pm 0.1$	$23.1^{EF} \pm 0.3$	$38.3^{KL} \pm 0.6$	$11.9^{B} \pm 0.2$	$0.4^{A} \pm 0.0$	$6.8^{H} \pm 0.4$	$1.4^{BCD} \pm 0.1$
4a	12	$1.5^{AB} \pm 0.1$	$23.4^{EF} \pm 0.4$	$38.7^{KL} \pm 0.5$	$12.1^{B} \pm 0.3$	$0.4^{A} \pm 0.0$	$5.4^{G} \pm 0.3$	$1.3^{BCD} \pm 0.1$
2b	12	$3.8^{EF} \pm 0.2$	$24.8^{H} \pm 0.1$	$40.1^{LM} \pm 0.2$	$11.6^{B} \pm 0.1$	$0.6^{AB} \pm 0.0$	$3.5^{DEF} \pm 0.2$	$0.6^{AB} \pm 0.0$
3b	12	$4.2^{F} \pm 0.2$	$23.4^{EF} \pm 0.3$	$41.3^{M} \pm 0.4$	$12.8^{B} \pm 0.1$	$0.5^{A} \pm 0.0$	$4.0^{EF} \pm 0.2$	$0.5^{AB} \pm 0.0$
4b	12	$5.2^{G} \pm 0.5$	$24.4^{GH} \pm 0.3$	$42.0^{M} \pm 0.5$	$12.2^{B} \pm 0.4$	$0.5^{AB} \pm 0.0$	$2.7^{CD} \pm 0.2$	$0.4^{AB} \pm 0.0$
5a	53	$1.5^{AB} \pm 0.0$	$24.3^{GH} \pm 0.1$	$28.5^{J} \pm 0.3$	$21.5^{F} \pm 0.2$	$0.9^{ABC} \pm 0.0$	$4.1^{F} \pm 0.1$	$1.2^{BC} \pm 0.0$
5b	53	$2.4^{C} \pm 0.1$	$24.4^{H} \pm 0.1$	$28.5^{J} \pm 0.2$	$22.6^{G} \pm 0.2$	$0.9^{ABC} \pm 0.0$	$3.6^{EF} \pm 0.1$	$0.9^{B} \pm 0.0$
6a	14	$0.7^{A} \pm 0.0$	$21.5^{D} \pm 0.1$	$18.3^{AB} \pm 0.2$	$26.8^{J} \pm 0.3$	$0.9^{ABC} \pm 0.1$	$6.8^{H} \pm 0.2$	$6.8^{G} \pm 0.2$
7a	14	$0.8^{A} \pm 0.0$	$18.4^{C} \pm 0.2$	$17.0^{A} \pm 0.2$	$23.3^{GH} \pm 0.2$	$9.9^{F} \pm 0.6$	$3.9^{EF} \pm 0.1$	$9.0^{H} \pm 0.3$
8a	14	$0.8^{A} \pm 0.0$	$17.7^{C} \pm 0.2$	$17.5^{A} \pm 0.3$	$33.2^{L} \pm 0.7$	$0.4^{A} \pm 0.0$	$8.6^{J} \pm 0.4$	$5.7^{F} \pm 0.3$
9a	14	$0.8^{A} \pm 0.0$	$20.8^{D} \pm 0.2$	$21.1^{CD} \pm 0.2$	$15.7^{C} \pm 0.3$	$0.9^{ABC} \pm 0.1$	$3.6^{EF} \pm 0.1$	$18.5^{K} \pm 0.4$
6b	14	$2.3^{BC} \pm 0.1$	$21.8^{D} \pm 0.4$	$24.1^{FH} \pm 0.4$	$29.4^{K} \pm 0.4$	$1.9^{C} \pm 0.2$	$3.1^{DE} \pm 0.2$	$3.4^{E} \pm 0.2$
7b	14	$2.7^{CD} \pm 0.2$	$13.7^{A} \pm 0.1$	$19.6^{BC} \pm 0.2$	$24.8^{H} \pm 0.1$	$24.8^{H} \pm 0.4$	$1.6^{B} \pm 0.1$	$3.3^{E} \pm 0.2$
8b	14	$3.0^{CDE} \pm 0.1$	$13.8^{A} \pm 0.2$	$22.5^{EF} \pm 0.2$	$46.4^{M} \pm 0.3$	$0.8^{ABC} \pm 0.0$	$3.2^{DE} \pm 0.2$	$1.9^{CD} \pm 0.1$
9b	14	$2.7^{CD} \pm 0.1$	$17.6^{C} \pm 0.1$	$25.4^{H} \pm 0.3$	$20.1^{E} \pm 0.1$	$1.3^{ABC} \pm 0.0$	$1.7^{B} \pm 0.1$	$12.1^{J} \pm 0.2$
10	48	$8.0^{J} \pm 0.2$	$21.4^{D} \pm 0.1$	$46.0^{N} \pm 0.2$	$6.9^{A} \pm 0.1$	$1.6^{C} \pm 0.0$	$1.0^{A} \pm 0.0$	$2.1^{D} \pm 0.1$
11	16	$3.6^{DEF} \pm 0.2$	$17.6^{C} \pm 0.2$	$21.8^{DE} \pm 0.4$	$15.6^{C} \pm 0.1$	$3.0^{D} \pm 0.6$	$0.7^{A} \pm 0.0$	$22.3^{M} \pm 0.6$
12	16	$3.8^{EF} \pm 0.2$	$15.9^{B} \pm 0.2$	$20.4^{CD} \pm 0.2$	$15.8^{C} \pm 0.3$	$11.4^{G} \pm 0.6$	$0.6^{A} \pm 0.0$	$19.4^{KL} \pm 0.4$
13	16	$3.8^{EF} \pm 0.2$	$15.9^{B} \pm 0.1$	$21.7^{DE} \pm 0.3$	$23.0^{G} \pm 0.5$	$2.8^{D} \pm 0.0$	$0.7^{A} \pm 0.0$	$19.6^{L} \pm 0.7$
14	16	$3.9^{EF} \pm 0.2$	$15.8^{B} \pm 0.2$	$21.4^{CD} \pm 0.2$	$20.3^{EF} \pm 0.2$	$7.2^{E} \pm 0.1$	$0.7^{A} \pm 0.0$	$18.5^{K} \pm 0.3$

[a] Means with different superscripts in columns differ significantly ($P < 0.01$). [b] 1, male chickens fed a commercial feed mixture, taken at 43 days of age within live weight range as broad as possible (1100–2500 g) (a, breast meat; b, thigh meat); 2, female chickens fed restrictively to the age of 87 days a diet based on wheat (a, breast meat; b, thigh meat); 3, female chickens fed restrictively to the age of 90 days a diet based on maize (a, breast meat; b, thigh meat); 4, female chickens fed semi ad libitum to the age of 74 days a diet based on maize (a, breast meat; b, thigh meat); 5, male turkeys fed a commercial feed mixture, taken at 140 days of age within live weight as broad as possible (14–24 kg) (a, breast meat; b, thigh meat); 6–9, female + male turkeys taken at the age of 56 days, fed either a commercial diet (control; 6) or a commercial diet to which linseed oil (7), sunflower oil (8), or fish oil (9), respectively, was added in the amount of 5% of fresh matter (a, breast meat; b, thigh meat); 10, female + male carps fed a diet based on wheat, taken at the age of 36 months within live weight as broad as possible (1470–2560 g), fillets; 11–14, female + male rainbow trouts fed either a commercial feed mixture (control; 11) or the same mixture to which linseed oil (12), sunflower oil (13), or linseed oil plus sunflower oil in the same ratio (14), respectively, was added in the amount of 5% of fresh matter, fillets. [c] Total lipids extracted by a hexane/2-propanol mixture (g/100 g of tissue). [d] g/100 g of the sum of total determined fatty acids. [e] Eicosapentaenoic acid (C20:5n–3) + docosahexaenoic acid (C22:6n–3).

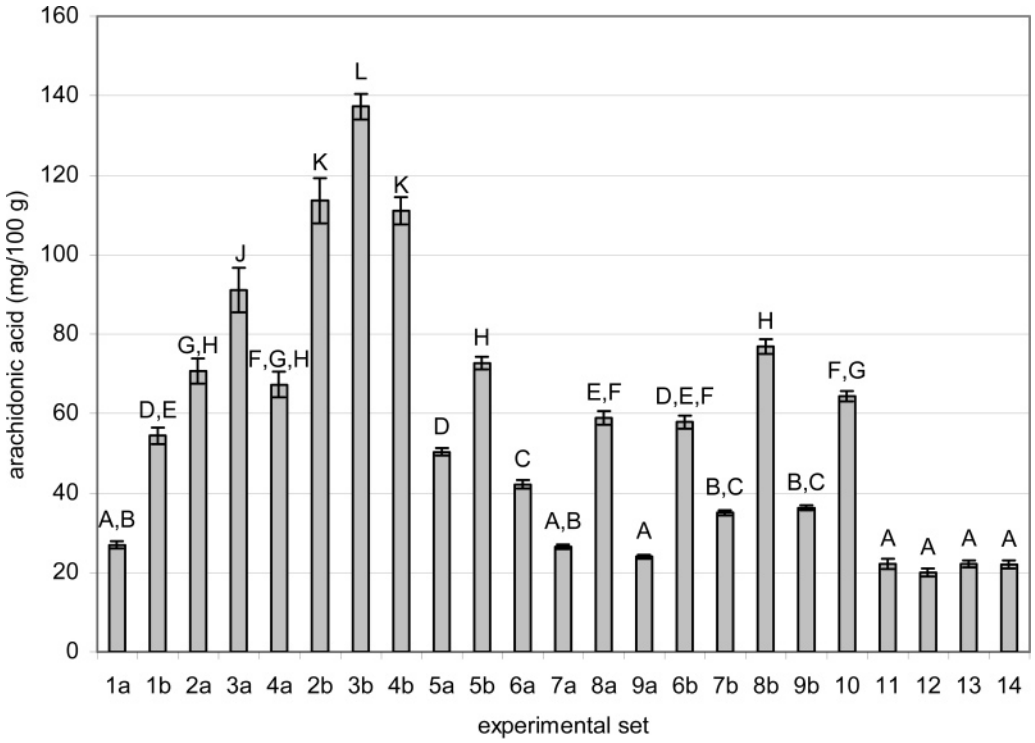

Figure 1. Arachidonic acid content in breast (a) and thigh meat (b) of male chickens fed a commercial feed mixture and slaughtered at the age of 43 days ($n = 49$; set 1); female chickens fed restrictively to the age of 87 days a diet based on wheat ($n = 12$; set 2); female chickens fed restrictively to the age of 90 days a diet based on maize ($n = 12$; set 3); female chickens fed semi ad libitum to the age of 74 days a diet based on maize ($n = 12$; set 4); male turkeys fed by a commercial feed mixture to the age of 140 days ($n = 53$; set 5); turkeys (the same number of males and females) taken at the age of 56 days fed either a commercial diet ($n = 14$; set 6) or a commercial diet with added linseed oil ($n = 14$; set 7), sunflower oil ($n = 14$; set 8), or fish oil ($n = 14$; set 9), respectively, in the amount of 5% of fresh matter; in fillets of carps (*C. carpio*) fed a diet based on wheat, taken at the age of 36 months ($n = 48$, the same number of males and females; set 10); in fillets of rainbow trouts (*O. mykiss*; without distinguishing sex) fed either a commercial feed mixture ($n = 16$; set 11) or the same mixture with added linseed oil in the amount of 5% of the fresh matter ($n = 16$; set 12), sunflower oil in the amount of 5% of fresh matter ($n = 16$; set 13), or linseed oil (2.5% of the fresh matter) plus sunflower oil (2.5% of the fresh matter; $n = 16$; set 14), respectively; A–L, means with different letters differ significantly ($P < 0.01$).

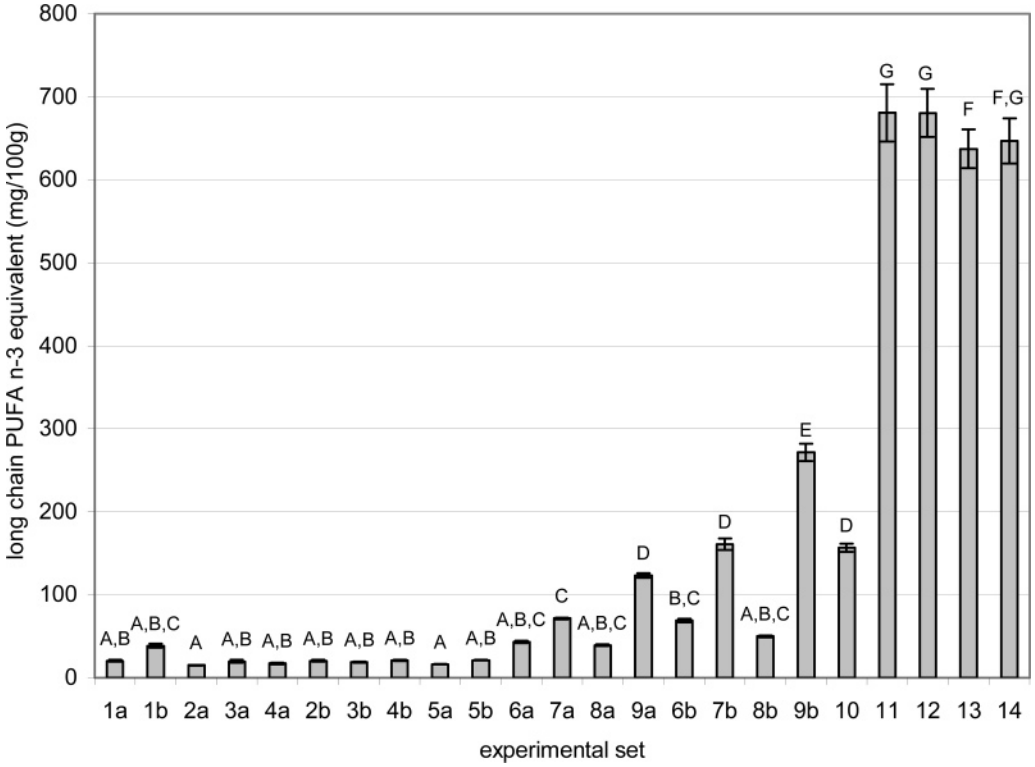

Figure 2. Content of long-chain (LC) n–3 polyunsaturated fatty acids (calculated as LC equivalent, LCE = 0.15 α-linolenic acid + eicosapentaenoic acid + docosahexaenoic acid; all components in mg/100 g of meat) in breast (a) and thigh meat (b) and fillets, respectively, of chickens, turkeys, carps (*C. carpio*), and rainbow trouts (*O. mykiss*), respectively; sets 1–14, see **Figure 1**; A–G, means with different letters differ significantly ($P < 0.01$).

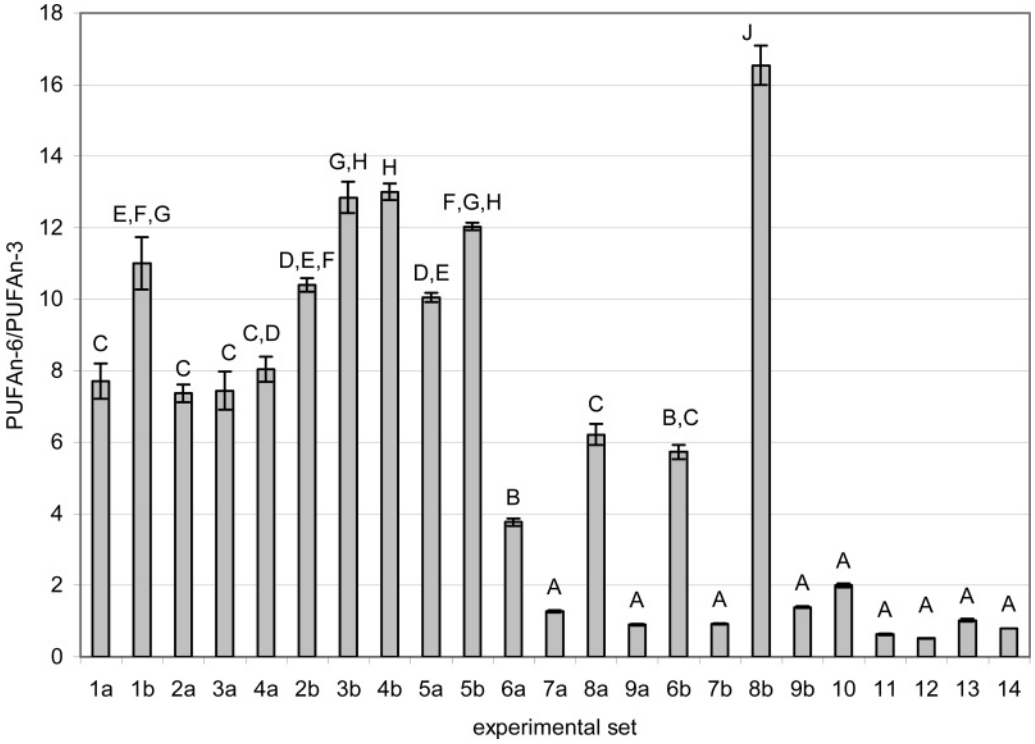

Figure 3. Polyunsaturated fatty acid (PUFA) n–6/n–3 ratio in breast (a) and thigh meat (b) and fillets, respectively, of chickens, turkeys, carps (*C. carpio*), and rainbow trouts (*O. mykiss*), respectively; sets 1–14, see **Figure 1**; A–J, means with different letters differ significantly ($P < 0.01$).

content and EPA + DHA content are expressed as a percentage of all determined fatty acids in **Table 3**.

Apart from an effect of animal species or inclusion of a component in the diet, which substantially change the fatty acid composition of this diet (e.g., plant oil), fatty acid content in the animal tissue can be also influenced by the unintentional changes in composition of a given commercial feed mixture. This was demonstrated in the chickens (set 1) taken in four cycles of commercial fattening. Only average values within the whole set are presented regarding feed mixture composition (**Table 2**) and fatty acid content in the tissues (**Table 3**; **Figures 1−3**). However, the PUFA n−6/PUFA n−3 ratio in the diet

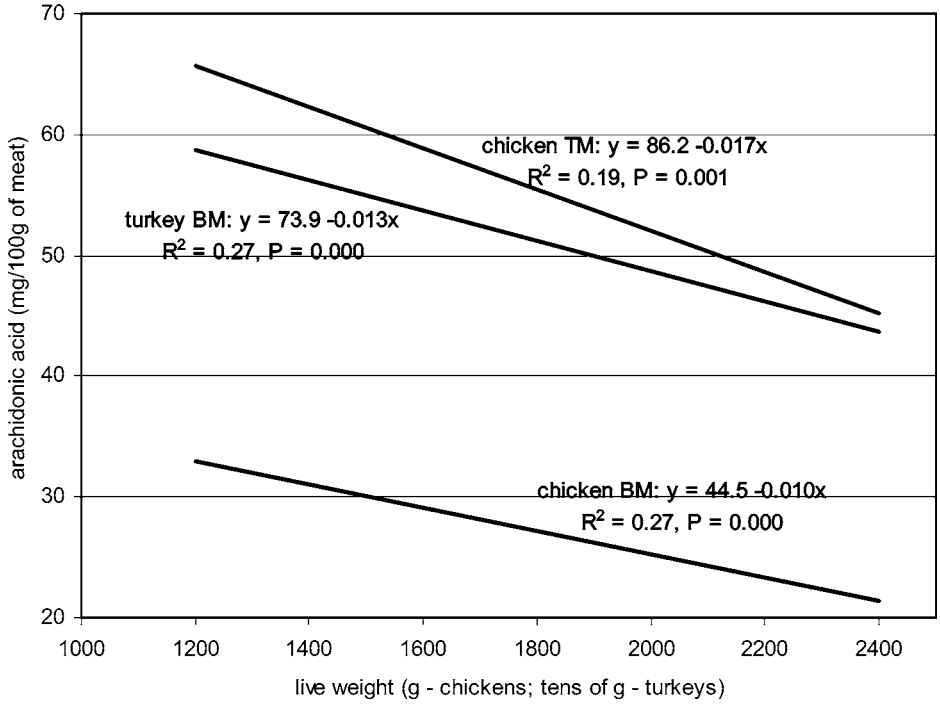

Figure 4. Dependence of arachidonic acid content in breast meat (BM) and thigh meat (TM) of male chickens fed a commercial feed mixture and slaughtered at the age of 43 days ($n = 49$) and in BM of male turkeys fed a commercial feed mixture and slaughtered at the age of 140 days ($n = 53$), respectively, on slaughter live weight.

was in the range of 5.2−41.0, and consequently AA content in the chicken BM and TM was in the range of 24.5−34.5 mg/100 g ($P < 0.01$) and 49.6−62.9 mg/100 g ($P < 0.01$), respectively. Corresponding LCE values in BM and TM were 9.4−29.1 mg/100 g ($P < 0.01$) and 12.0−24.8 mg/100 g ($P < 0.01$), respectively. When recalculated to the same basis, LCE content in BM and TM of the retail chicken samples derived from the data of ref *16*, 13.5 and 20.7 mg/100 g, respectively, was well within the above-mentioned range.

AA content in BM and TM of chickens fed the commercial feed mixture (1a and 1b in **Figure 1**) was substantially lower in comparison with the corresponding data of ref *16* [64 and 106 mg of AA/100 g in the retail BM and TM samples] but was comparable in the case of turkey TM. Also, ref *23* reported higher AA percentage in BM and TM of chickens fed a standard diet (5.0 and 3.7%) than found in the present experiment (1a and 1b in **Table 3**). On the other hand, our results regarding chicken BM (1a in **Figure 1**) are in agreement with the data of ref *18*: 31 mg/100 g. Li et al. (*17*) reported AA content in lean meat of different species to be in the range of 30−99 mg/100 g, which is similar to our results.

A similar effect of an inconsistent composition of a common commercial feed mixture (sets 5 and 6, **Table 2**) on fatty acid content in the tissues was found in the present experiment also in the case of turkeys. An inclusion of some fish product in the feed mixture within set 6 (indicated by >3 higher EPA + DHA percentage; **Table 2**) could be supposed. AA content (**Figure 1**) in both BM (5a and 6a) and TM (5b and 6b) of turkeys within set 5 was significantly ($P < 0.01$) higher in comparison with set 6. LCE content tended to be higher in turkey BM and TM in set 6 in comparison with set 5 (6a and 5a, and 6b and 5b, respectively, **Figure 2**), and the PUFA n−6/PUFA n−3 ratio in the tissues of 6a and 6b was more than twice lower in comparison with the tissues of 5a and 5b, respectively ($P < 0.01$; **Figure 3**).

Very high AA content was found (as far as breast meat is concerned in the present experiment) in chickens fed intentionally slowly to the higher age, especially in chickens fed restrictively the diet based on maize (AA content = 91 mg/100 g, set 3a in **Figure 1**). The probable reason was a very unfavorable PUFA n−6/PUFA n−3 ratio in the diet within set 3 (24.6, **Table 2**) and the longer duration of feeding. However, despite the very similar n−6/n−3 ratio in diet 8 (**Table 2**), the PUFA n−6/n−3 ratio in the TM of turkeys in set 8 (8b) was substantially higher ($P < 0.01$) in comparison with the chicken set 3 (3b; **Figure 3**). This could be explained by higher LA and lower LNA percentages in diet 8 and, consequently, by higher LA transfer to, and decreased synthesis of, n−3 PUFA in turkey TM, which likely could not be compensated for by a higher EPA + DHA percentage in the diet 8.

Arachidonic acid content ($P > 0.05$) as low as in fish fillets (both carp and rainbow trout; sets 10−14, **Figure 1**) was found in breast meat of turkeys fed the diet with fish oil (9a) and also in BM of turkeys fed the diet with linseed oil and, surprisingly, in BM of chickens fed the commercial feed mixture (1a). As far as AA content in rainbow trout is concerned, the data of ref *30*, 21 mg/100 g, and the present experiment (sets 11−14, **Figure 1**) are nearly identical. Barrado et al. (*29*) reported AA percentage in muscle (in the dependence on water temperature, water composition, vital space, stress, and nutrition) to be in the range of 0.36−0.69%, which also corresponds with our data (sets 11−14, **Table 3**). According to ref *31*, AA percentage in muscle samples of rainbow trout (0.4−0.6%) was influenced by the ration size necessary to obtain maximal growth. With regard to common carp, ref *30* reported an AA content (60 mg/100 g) very similar to our data (set 10 in **Figure 1**), and the values of ref *32* from Madagascar are lower (2.9−5.9% of total fatty acids; compare with set 10, **Table 3**).

Addition of sunflower oil (with a high content of linoleic acid and therefore a high PUFA n−6/PUFA n−3 ratio; set 8 in **Table 2**) in the diet increased ($P < 0.01$) AA content in turkey breast meat (59 mg/100 g; 8a in **Figure 1**) in comparison with turkeys fed the commercial feed mixture (5a and 6a, respectively, **Figure 1**) in the present experiment.

Arachidonic acid content in carp fillets (64 mg/100 g; set 10 in **Figure 1**) differed conspicuously ($P < 0.01$) from that in all rainbow trout fillets (sets 11−14) and was even higher ($P < 0.05$) than AA content in BM and TM of one of the sets of turkeys fed the commercial diet (6a, 6b) and the set of chickens fed the commercial diet (1a, 1b).

In comparison with AA content, the sets of corresponding poultry tissues were more homogeneous as far as LCE content in the present experiment is concerned. With regard to breast meat, only turkeys fed the diet with linseed oil deposited more ($P < 0.01$) LCE (71 mg/100 g; 7a in **Figure 2**) as compared to all other poultry sets except turkeys fed the diet with fish oil, which had still a higher ($P < 0.01$) content of LCE (123 mg/100 g; 9a in **Figure 2**). The above-mentioned value regarding linseed oil fed birds corresponds with the EPA + DHA percentage of 9.0% (set 7a in **Table 3**), which is a much higher figure in comparison with BM of chickens fed the diet enriched by 8.2% of linseed oil in the experiment of ref *24*: 2.1%. The likely reason is a nearly twice higher EPA + DHA content in the particular diet in the present experiment (1.1%, **Table 2**).

BM of turkeys fed the diet with fish oil and also TM of turkeys fed the diet with linseed oil (161 mg/100 g; 7b) did not differ in LCE content from carp fillets (157 g/100 g, set 10 in **Figure 2**) in the present experiment. Thigh meat of turkeys fed the diet with fish oil (9b) had an even higher ($P < 0.01$) LCE content (271 mg/100 g) than carp fillets. The latter value corresponds with the EPA + DHA percentage of 12.1% (9b, **Table 3**), which is by nearly 30% higher in comparison with TM of chickens fed the diet with 6% of fish oil in the experiment of ref *33*.

A >4 times lower ($P < 0.01$) LCE content in carp fillets in comparison with the sets of rainbow trout (sets 11−14, **Figure 2**) clearly reflects in the present experiment the differences in EPA + DHA contents of the particular diets (**Table 2**). The LCE content in carp fillets (157 mg/100 g) is somewhat lower and the values regarding rainbow trout (637−681 mg/100 g) higher than the figures found in ref *30*, recalculated to the same basis: 211 and 572 mg/100 g, respectively. Moreover, the EPA + DHA percentage in fillets of rainbow trout (reared in a freshwater pond) in the present experiment (sets 11−14, **Table 3**) corresponds more with the data of ref *34* for rainbow trout fed in seawater cages (22.5%) than with fish fed in a freshwater pond (26.2%).

Because cumulative feed intake during the finishing period of the fattening was measured in all experimental sets, it was possible to calculate an amount of individual fatty acids supplied by a diet and to compare this value with an amount of particular fatty acids deposited in 100 g of the tissue. The ratio of deposited long-chain n−3 PUFA equivalent (mg of LCE/100 g of tissue; **Figure 2**) to LNA + EPA + DHA provided by a diet within the fixed period of the feeding (g of LNA + EPA + DHA/30 days) was in the poultry sets in the range of 0.8 (BM of turkeys fed the commercial feed mixture; set 5a) to 14.8 (TM of turkeys fed the commercial feed mixture; set 6b). The latter value tended to be higher in comparison with TM of turkeys fed the diet with fish oil (a ratio of 11.0). The ratio in the set of rainbow trouts fed the diet with sunflower oil (285.0) was surprisingly higher (and more efficient transfer of n−3 PUFA from the diet to the tissue could therefore be supposed) than in the set of rainbow trout fed the diet with linseed oil (a ratio of 114.3; the values were not statistically evaluated, because only the means of the whole sets were considered). In the set of carps the ratio was 84.6.

As far as a healthy human nutrition is concerned, the PUFA n−6/PUFA n−3 ratio of the diet should be as close to 1 as possible (*14*). Fillets within all four rainbow trout sets met this criterion in the first place in the present experiment (sets 11−14, **Figure 3**). However, BM and TM of turkeys fed the diet with either fish oil (9a, 9b) or linseed oil (7a, 7b, **Figure 3**) did not differ in this trait from the rainbow trout fillets ($P > 0.05$). Although not statistically different ($P > 0.05$), the PUFA n−6/PUFA n−3 ratio in carp fillets (2.0, set 10 in **Figure 3**) tended to be higher in comparison with rainbow trout or the above-mentioned turkey tissues.

Because nowadays it is unrealistic to achieve the above optimal PUFA n−6/PUFA n−3 ratio in societies with the so-called Western-type of consumption, the nutritional commissions of particular countries recommend the higher value, most commonly 4−5 [e.g., the European population reference intake (*1*)]. Chicken breast meat (1a−4a, **Figure 3**) and especially BM of turkeys fed the commercial feed mixture within set 6 (6a in **Figure 3**) were not very different from this value in the present experiment. On the other hand, the PUFA n−6/PUFA n−3 ratio in chicken thigh meat, including thigh meat of turkeys within set 5, was substantially higher ($P < 0.01$; 1b−5b, **Figure 3**). The most unfavorable food ($P < 0.01$) from the above viewpoint was TM of turkeys fed the diet with sunflower oil (the ratio 16.5; 8b, **Figure 3**). These results confirm previous studies on the close relationship between dietary sunflower oil (high LA percentage) and PUFA n−6 content in poultry tissues (*35*, *36*).

General Assessment of Some Factors Influencing AA Content, EPA + DHA Content, and PUFA n−6/PUFA n−3 Ratio in the Tissues. The dependence of AA content in the tissue (Y, mg /100 g of the tissue) on LA content in the diet (X, % of total FA) was significant ($P < 0.0001$) both in the set of all analyzed chicken tissues ($Y = -218.2 + 6.91X$; $R^2 = 0.60$) and for all corresponding turkey tissues ($Y = 6.2 + 1.28X$; $R^2 = 0.48$). This finding seems contrary to the data of ref *4*, where, in accordance with the suggested strong metabolic regulation of the AA formation, no increase of AA in the human tissues with increasing dietary LA was reported. The latter statement was confirmed in the present experiment only in rainbow trout ($R^2 = 0.03$, $P = 0.28$; the corresponding relationship was not possible to evaluate in carp, because only one diet was used). However, it is difficult to compare the above relationships found in the present experiment and in ref *4* due to the probably different AA origin in the tissues: from plasma membrane phospholipids and from adipose tissue triglycerides, respectively.

The significant relationship between dietary LNA (X, % of total FA) and tissue EPA + DHA content (mg/100 g of the tissue) was found in the present experiment only in the set of all analyzed chicken tissues ($Y = 14.1 + 1.44X$, $R^2 = 0.03$, $P = 0.011$). Even this relationship was weak (R^2 was very low) and was significant only due to the sufficient number of measurements ($n = 84$; sets 1−4, **Table 1**); the relationships in turkey and rainbow trout tissues, respectively, were insignificant. This is a confirmation of the unreliable and restricted degree of LNA to EPA + DHA conversion (*37*). Also, according to ref *27*, supplementing the poultry diet with LNA does not result in a noticeable increase in EPA content in the edible tissues. However, data regarding other animal species indicate a different conversion rate of dietary LNA to long-chain n−3 PUFA: diets rich in LNA resulted in an increased level of EPA but failed to increase DHA in lamb, beef, and pork meat (*38*) or in hamster red blood cells (*39*).

When all tissues within all animal species evaluated in the present experiment were taken as a one set, the tissue PUFA

n−6/PUFA n−3 ratio (Y) increased significantly ($P < 0.001$) with an increase of dietary LA/LNA ratio (X) in all tissues (except carp fillet, which was not possible to evaluate; see above) according to the equation $Y = 2.0 + 0.41X$ ($R^2 = 0.68$).

Higher absolute values of arachidonic acid content in thigh meat in comparison with breast meat ("a" columns vs corresponding "b" columns in **Figure 1**) were a consequence of higher fat content in TM both in chickens and in turkeys (**Table 3**). Moreover, the highest AA content found in the present experiment (138 mg/100 g; 3b in **Figure 1**) was in TM of chickens fed restrictively to the age of 90 days the diet based on maize (fat content in the tissue of 4.2%). However, when expressed on the relative basis as a percentage of total determined fatty acids, AA (Y, %) decreased significantly ($P < 0.001$) with increasing fat content (X, %) in all evaluated tissues. The overall regression (all tissues within all animal species evaluated as a one set, $n = 500$) was $Y = 4.7-0.54X$ ($R^2 = 0.41$). The same dependence was observed in our previous experiment regarding cholesterol content in meat of some fish and poultry species (*40*). In the case of arachidonic acid, the above relationship is possible to explain on the basis of the data of ref *41*, which reported a much higher linoleic acid/α-linolenic acid ratio in the lean meat than in meat with a higher fat level, because LA (a metabolic precursor of arachidonic acid in the animal tissues) is preferably deposited in membrane phospholipids as compared to a storage triacylglycerol fraction.

Similarly, the EPA + DHA percentage in the tissue (Y, %) decreased significantly ($P < 0.001$) in all tissues with increasing fat content in the tissue in the present experiment. The overall regression (when all animal species and all tissues were taken as a one set) was in the form $Y = 6.0 - 0.33X$ ($R^2 = 0.35$). In this case the finding is contrary to the data of ref *41*, which suggested more equal partitioning of α-linolenic acid (a precursor of long-chain PUFA n−3) between storage triacylglycerols and membrane phospholipids on the one hand and not negligible de novo synthesis of longer chain fatty acids of the n−3 series in the membranes on the other hand, with a probable consequence of a weak relationship between fat content and EPA + DHA content in the muscle tissue. On the other hand, in accordance with the data of ref *41*, no dependence of the PUFA n−6/PUFA n−3 ratio, but a significant dependence of the linoleic/α-linolenic ratio (Y), on the fat content in the tissue (X, %) was found in the present experiment when all species and all tissues were taken as one set: $Y = 32.2 - 6.54X + 0.4251X^2$ ($R^2 = 0.18$, $P < 0.001$).

The animals within the experimental sets 1 (chickens), 5 (turkeys), and 10 (carps), respectively, were taken within the live weight range as broad as possible in order to evaluate an effect of the growth intensity (live weight in the fixed, i.e., slaughter, age) on fatty acid content. Despite using commercial breeds of chickens and turkeys with balanced growth characteristics and overall small live weight variance, it was possible to select a representative set of the extreme specimens due to the large numbers of birds in the fattening house (30000 in the case of chickens).

The arachidonic acid content in chicken and turkey BM and in turkey TM decreased linearly with increasing live weight at slaughter age (43 and 140 days, respectively; **Figure 4**). Inclusion of the quadratic term was not significant in any case. This mechanism could also explain the higher ($P < 0.01$) AA content in BM and TM of chickens within set 3 in comparison with set 4 (**Figure 1**). Diet 3 was quantitatively and qualitatively restrictive (**Table 1**), and the chickens within set 3 grew less intensively (reaching slaughter live weight by 16 days later) as compared to the chickens within set 4, which were offered the diet of an identical composition but fed semi ad libitum. Similarly, ref 42 found a lower AA percentage in meat of rabbits selected for the growth rate (1.77%) in comparison with control (2.10%). The AA content in turkey TM and carp fillets was not influenced by varying growth intensity in the present experiment ($P > 0.05$).

The deposition of LCE (Y; mg/100 g) in breast meat of poultry differing in live weight at slaughter age (X) was different between chickens (increase: $Y = 8.3 + 0.008X$, $R^2 = 0.11$, $P = 0.038$; X in g) and turkeys (decrease: $Y = 24.9 - 0.005X$, $R^2 = 0.24$, $P = 0.000$; X in tens of g). In chicken TM, turkey TM, and carp fillets, respectively, LCE content was not influenced by live weight at slaughter age ($P > 0.05$).

It could be concluded that PUFA content in the animal products depends not only on an animal species, a given tissue, or manipulation of the diet composition but also on the unintentional seasonal changes in the composition of a "standard" commercial feed mixture. Furthermore, when expressed on the relative basis, both AA percentage and EPA + DHA percentage of total determined fatty acids decreased significantly with increasing fat content in all evaluated tissues in the present experiment.

On the basis of the suggestion of ref *15* that an anti-inflammatory diet should provide an AA intake of <90 mg/day and supposing a usual poultry or fish meat portion of 200 g, the following food samples examined in the present experiment met the criterion of healthy nutrition: rainbow trout fillets, BM of chickens fed the commercial diet, BM of turkeys fed the commercial diet or the diet with LO and FO, respectively, and TM of turkeys fed the diet with LO or FO. On the other hand, considering the assumption that EPA + DHA intake should be optimally as high as 1.25 g/day (*43*), only rainbow trout fillet approached the criterion of recommendable food from this viewpoint.

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