

Effect of diet, sex and age on fatty acid metabolism in broiler chickens: *n*-3 and *n*-6 PUFA

R. Poureslami¹, K. Raes^{1†}, G. M. Turchini², G. Huyghebaert³ and S. De Smet^{1*}

¹Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

²School of Life and Environmental Sciences, Deakin University, Warrnambool, Vic, Australia

³Animal Science Unit, Institute for Agricultural and Fisheries Research, Scheldeweg 68, 9090 Melle, Belgium

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The PUFA metabolism in broiler chicken was studied through the whole body fatty acid balance method. Four dietary lipid sources (palm fat, Palm; soyabean oil, Soya; linseed oil, Lin; fish oil, Fish) were added at 3% to a basal diet containing 5% palm fat. Diets were fed to female and male birds from day 1 to either day 21 or day 42 of age. Birds fed the Lin diet showed a significantly higher 18:2*n*-6 accumulation compared with the other diets (85.2 v. 73.6% of net intake), whereas diet did not affect 18:3*n*-3 accumulation (mean 63% of net intake). Bioconversion of 18:2*n*-6 significantly decreased in the order Palm > Lin > Soya > Fish (4.7, 3.9, 3.4 and 1% of net intake, respectively). The 18:3*n*-3 bioconversion on the Palm and Soya diets was similar and significantly higher than in broilers on the Lin diet (9.1 v. 5.8% of net intake). The β -oxidation of 18:2*n*-6 was significantly lower on the Lin diet than on the other diets (10.8 v. 23.3% of net intake), whereas β -oxidation of 18:3*n*-3 was significantly higher on the Fish diet than on the other diets (41.5 v. 27.3% of net intake). Feeding fish oil suppressed apparent elongase and desaturase activity, whereas a higher dietary supply of 18:3*n*-3 and 18:2*n*-6 enhanced apparent elongation and desaturation activity on the PUFA involved in the *n*-3 and *n*-6 pathway, respectively. Accumulation of 18:2*n*-6 and 18:3*n*-3 increased and β -oxidation decreased with age. Sex had a marginal effect on the PUFA metabolism.

PUFA: Broiler chickens: Elongation: Desaturation: Oxidation

In chickens, as with all animals, body fatty acids are derived from dietary uptake, *de novo* synthesis and/or bioconversion. Among the different fatty acid classes, *n*-3 long-chain PUFA (*n*-3 LCPUFA) are of particular interest due to their beneficial role on human health⁽¹⁾. In vertebrates, α -linolenic acid (18:3*n*-3) and linoleic acid (18:2*n*-6) cannot be biosynthesised *de novo*, but are derived from the diet and subsequently can be bioconverted to longer and more unsaturated *n*-3 and *n*-6 LCPUFA, respectively. This *in vivo* bioconversion of *n*-3 LCPUFA includes endoplasmic Δ -6 desaturation, chain elongation and Δ -5 desaturation of the precursor 18:3*n*-3 to EPA (20:5*n*-3), which is subsequently converted to docosapentaenoic acid (22:5*n*-3) by chain elongation. The final metabolite, DHA (22:6*n*-3), is synthesised by chain elongation, Δ -6 desaturation and peroxisomal β -oxidation of 22:5*n*-3⁽²⁾. The *n*-6 pathway involves the same enzymes and conversion steps as the *n*-3 pathway, with arachidonic acid (20:4*n*-6) being the major metabolite of dietary 18:2*n*-6.

The absolute amount of 18:3*n*-3 intake is of prime importance to the efficiency of conversion to the LCPUFA⁽³⁾. However, the production of *n*-3 LCPUFA, particularly 22:6*n*-3, from 18:3*n*-3 is limited in the human body^(4,5). Isotopic

tracer studies showed that the conversion of 18:3*n*-3 to 22:6*n*-3 is about 1% in infants and substantially lower in adults with, however, important differences between men and women^(4,5). Therefore, it is often postulated that the *n*-3 LCPUFA are semi-essential and should be sufficiently provided by the diet. In order to optimise the fatty acid composition of foods derived from farmed animals, knowledge of their LCPUFA metabolism is required. To study PUFA metabolism, several *in vivo* and *ex vivo* methods have been applied in different species. Isolation of cells or tissue microsomes and incubation with labelled fatty acids is a common *ex vivo* method⁽⁶⁾. Besides the need for sophisticated analyses and expensive reagents, other drawbacks of the *ex vivo* methods are that some controlling factors are missing and that this approach is restricted to one tissue⁽⁷⁾. Therefore, to gain an accurate insight into the whole body (WB) metabolism of PUFA, a proper *in vivo* method is desired. Different *in vivo* techniques appropriate to WB fatty acid metabolism have been detailed elsewhere⁽⁸⁾. Recently, a WB fatty acid balance method has been suggested as a reliable method to study the overall PUFA metabolism in fish⁽⁷⁾. The WB fatty acid balance method comprises a feeding experiment, quantification

Abbreviations: Fish, fish oil diet; LCPUFA, long-chain PUFA; Lin, linseed oil diet; Palm, palm fat diet; Soya, soyabean oil diet; WB, whole body.

* **Corresponding author:** Stefaan De Smet, fax +32 9 264 9099, email stefaan.desmet@ugent.be

† Present address: Research Group EnBiChem, Department of Industrial Engineering and Technology, University College West-Flanders, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium

of the initial and final WB fatty acid composition and the excreted fatty acids. Thereafter, the partitioning of fatty acids among excretion, accumulation, conversion to longer chain and more unsaturated metabolites and mitochondrial β -oxidation is computed. To the best of our knowledge, no research has examined the different fates of n -3 and n -6 PUFA metabolism (accumulation, conversion to longer chain and more unsaturated metabolites and mitochondrial β -oxidation) in poultry simultaneously by a WB approach. The rationale for the present study was to quantify the fate of individual dietary fatty acids in broiler chickens as a function of the dietary fatty acid source, and the animal factors age and sex through a WB fatty acid balance method.

Materials and methods

Animals and diets

The experiment was carried out according to the guidelines of the Ethics Committee of Ghent University (Belgium) for the humane care and use of animals in research. A total of 400 1 d-old chickens of the Ross308 strain were obtained from a local hatchery (Belgabriod, Merksplas, Belgium). The birds were randomly allotted to eight floor pens with fifty males or females per pen, with each pair of pens randomly selected to receive either one of the four experimental diets. The experiment was accomplished in two periods of age (days 7–21 and days 21–42). On day 7 and day 21, the birds were individually weighed, and six birds per pen around the average live weight were selected and placed in metabolic cages with two birds of the same sex per cage and three replicate cages per diet \times sex combination. Birds in the cages received the same diets as in the pens. Evolution of live weight gain was comparable in the cages and in the pens. Feed and water were provided *ad libitum*. Feed intake and body weight were recorded for the caged birds during the respective period of study. Four diets based on wheat and soyabean meal and differed only in the fat source were fed (Table 1): palm fat (Palm, 8% palm fat, SFA source); soyabean oil (Soya, 5% palm fat + 3% soyabean oil, 18:2n-6 source); linseed oil (Lin, 5% palm fat + 3% Lin, 18:3n-3 source); Fish oil (Fish, 5% palm fat + 3% fish oil, n -3 LCPUFA source). The fatty acid profile of the diets is given in Table 2. Differences between diets for average live weight at the start and end of the two periods were minimal, except for a somewhat lower average live weight on day 21 and day 42 for the Fish diet.

Sample collection and sample preparation

On days 21 and 42, after overnight fasting, caged birds were killed by cervical dislocation. Since the present study was part of a larger study, data from the analysis of separate anatomical compartments were used and pooled. Blood was collected, and the carcasses were dissected in seven body compartments: (1) complete deboned skinless thigh muscle; (2) skinless breast muscle; (3) liver; (4) heart; (5) brain; (6) abdominal fat pad (fat surrounding cloaca and adjacent muscles); (7) blood and carcass trimmings, i.e. skin, wings, feet, bones, neck, offal, feathers, visceral organs and remainders, named the rest compartment. The parts composing

Table 1. Composition of the basal experimental diet

Ingredients	g/kg
Wheat	580
Soyabean meal (48% CP)	299
Added fat	80.0
Dicalcium phosphate	12.0
Calcium carbonate	7.20
Salt	2.90
Sodium bicarbonate	0.60
L-Lys monohydrochloride	3.00
L-Thr	1.40
DL-Met	2.60
R Phytase*	0.20
BFW enzyme†	0.40
Vitamin and mineral premix‡	10.0
Calculated composition (fresh weight basis)	
ME _n (MJ/kg)	12.6
CP (g/kg)	205
Crude fat (g/kg)	98.6

CP, crude protein.

* Exogenous phytase from Ronozyme P-5000.

† Bio Feed Wheat enzymes; NSP enzyme preparation from Ronozyme WX.

‡ The vitamin/mineral premix provided the following quantities (mg/kg of diet): retinyl acetate 4; cholecalciferol 0.07; DL- α -tocopheryl acetate 50; menadione 2.5; thiamin 2.2; choline chloride 650; riboflavin 7.5; pantothenic acid 38; pyridoxine 5.5; cyanocobalamin 0.035; nicotinic acid 13; biotin 0.20; folic acid 1; ethoxyquin 35; butylated hydroxytoluene 25; iodine 2; Co 1; Se 0.4; Cu 25; Mn 60; Zn 70; Fe 45.

the rest compartment were minced together in a 20 litre three-bladed cutter for 10 min (Moled HK20/L, REX-MASCIINEN, Pinneberg, Germany). Samples per compartment were pooled for the two birds per cage, weighed, vacuum packed and stored at -20°C until analysis. From days 7 to 21 and days 21 to 42, faeces were totally collected from the cages, weighed, homogenised, and a representative sample from each cage was freeze-dried and stored at -20°C until analysis. Information on the fatty acid composition of different body compartments can be found in a recent report by Poureslami *et al.* ⁽⁹⁾.

Fatty acid analyses

Lipids were extracted from feed, body compartments and excreta using the method of Folch *et al.* ⁽¹⁰⁾ upon mincing and homogenisation. Samples were then transmethylated,

Table 2. Fatty acid profile of the diets

Fatty acid (mg/g feed)	Palm	Soya	Lin	Fish
18:2n-6	14.7	24.1	14.5	12.3
18:3n-6	0.02	0.01	0.01	0.10
20:3n-6	–	–	–	0.03
20:4n-6	–	–	–	0.24
18:3n-3	0.86	2.38	14.1	1.27
18:4n-3	0.02	0.02	0.02	0.74
20:5n-3	–	–	–	4.11
22:5n-3	–	–	–	0.44
22:6n-3	–	–	–	3.03
Σ SFA	31.4	23.9	21.2	21.1
Σ MUFA	0.17	0.17	0.15	0.38
Total fatty acids	89.5	79.9	76.1	79.4

Palm, palm fat diet; Soya, soyabean oil diet; Lin, linseed oil diet; Fish, fish oil diet; –, not detected; Σ SFA, sum of SFA; Σ MUFA, sum of MUFA.

and fatty acid methyl esters were analysed by GC (HP6890, Brussels, Belgium) on a CPSil88 column for fatty acid methyl esters (100 m × 0.25 mm × 0.2 μm, Chrompack, Middelburg, The Netherlands) according to the method described by Raes *et al.* (11). Fatty acids were identified by comparing their retention times with those of the corresponding standards (Sigma, Brussels, Belgium) Nonadecanoic acid was used as internal standard to obtain quantitative data of the fatty acid contents (mg/100 g tissue). Before WB fatty acid balance calculations, a theoretical correction was made for fatty acid methyl esters based on the response correction factors discussed by Ackman (12).

Whole body fatty acid balance calculations

The different fates (excretion, body accumulation, elongation, desaturation and mitochondrial β-oxidation) of dietary 18:2*n*-6 and 18:3*n*-3 were calculated according to the method described in detail by Turchini *et al.* (7,13) and Turchini & Francis (14). Summarising the methodology, the WB fatty acid balance was computed in distinct steps. Initially, the fatty acids in the diets, faeces and the initial and final carcasses (sum of fatty acid mass in seven body compartments) were quantified in mg. Knowing the fatty acid mass ingested and excreted, apparent digestibility of individual fatty acids was computed, and the net fatty acid intake (fatty acid absorption) was calculated. The difference between fatty acid accumulation in the WB and the net intake was considered as the total appearance or disappearance of a specific fatty acid. Next, the balance of *n*-3 and *n*-6 PUFA was computed. The quantity of fatty acid was first converted from mg to μmol of appeared/disappeared fatty acid per chicken WB. Then a backward calculation was made along each of the fatty acid metabolic pathways. The number of μmol of a longer chain or more unsaturated fatty acid that appeared was subtracted from the number of μmol of the previous fatty acid in the pathway. This allowed quantifying the bio-conversion (i.e. elongation and/or desaturation) of an individual fatty acid expressed as μmol of fatty acid/g body weight per day. Fatty acid β-oxidation was subsequently computed after all computations along the pathway. In four cases (out of forty-eight), a small negative β-oxidation value was obtained (i.e. an appearance of 18:2*n*-6 or 18:3*n*-3). These values were turned from a negative value to zero before further calculation. It was verified that this did not affect the significance of the treatment effects. The equations and details for calculating the fate of individual fatty acids (% *ex novo* production, % body accumulation, % bioconversion, % β-oxidation) were previously described (14).

Data analyses

The data were analysed using S-PLUS for Windows (version 6.1; Insightful, Seattle, WA, USA). A linear model was used to analyse the fixed effects of diet, age and sex and their interaction terms. In case of a significant diet effect, the mean values were compared with the Tukey's *post hoc* test (*P*<0.05).

Table 3. *n*-3 and *n*-6 PUFA content of whole body broiler chickens

Fatty acid (mg/g broiler chicken)	Diet (n 12)				Age (n 24)		Sex (n 24)		P			
	Palm	Soya	Lin	Fish	7-21 d	21-42 d	Male	Female	Diet	Age	Sex	Interactions*
18:2 <i>n</i> -6	11.0 ^c	21.5 ^a	13.7 ^b	9.77 ^c	11.1	16.9	13.6	14.4	<0.001	<0.001	0.136	D × A
18:3 <i>n</i> -6	0.15 ^{b,c}	0.16 ^b	0.20 ^a	0.14 ^c	0.15	0.17	0.15	0.16	<0.001	<0.001	0.189	—
20:3 <i>n</i> -6	0.13 ^b	0.15 ^a	0.12 ^{b,c}	0.12 ^c	0.12	0.14	0.12	0.13	<0.001	<0.001	0.097	—
20:4 <i>n</i> -6	0.60 ^b	0.85 ^a	0.41 ^c	0.45 ^c	0.53	0.62	0.57	0.59	<0.001	<0.001	0.470	—
18:3 <i>n</i> -3	0.51 ^c	1.86 ^b	12.1 ^a	0.84 ^c	3.07	4.62	3.76	3.92	<0.001	<0.001	0.464	D × A
18:4 <i>n</i> -3	0.04 ^c	0.05 ^c	0.18 ^b	0.48 ^a	0.15	0.22	0.18	0.20	<0.001	<0.001	0.045	D × A, A × S
20:5 <i>n</i> -3	0.03 ^c	0.06 ^c	0.43 ^b	2.55 ^a	0.62	0.92	0.74	0.79	<0.001	<0.001	0.322	D × A
22:5 <i>n</i> -3	0.05 ^d	0.13 ^c	0.42 ^b	1.02 ^a	0.33	0.48	0.40	0.41	<0.001	<0.001	0.570	D × A
22:6 <i>n</i> -3	0.05 ^c	0.11 ^{c,d}	0.26 ^b	2.06 ^a	0.51	0.73	0.60	0.64	<0.001	<0.001	0.275	D × A
Total fatty acid†	89.6	99.1	99.4	87.4	76.7	111	89.8	98.0	0.051	<0.001	0.032	—

Palm, palm fat diet; Soya, soyabean oil diet; Lin, linseed oil diet; Fish, fish oil diet; RMSE, root mean squares error; D, diet; A, age; S, sex.

a,b,c,d Mean values within a row with unlike superscript letters were significantly different (*P*<0.05).

* Significant interaction terms at *P*>0.05.

† Sum of SFA, MUFA and PUFA.

Table 4. *n*-3 and *n*-6 PUFA intake, apparent digestibility, appearance/disappearance, accumulation, bioconversion and β-oxidation in broiler chickens

	Diet (<i>n</i> 12)				Age (<i>n</i> 24)		Sex (<i>n</i> 24)		RMSE	<i>P</i>			
	Palm	Soya	Lin	Fish	7–21 d	21–42 d	Male	Female		Diet	Age	Sex	Interactions*
Intake (mg/d)													
18:2 <i>n</i> -6	1552 ^b	2472 ^a	1555 ^b	1252 ^c	1026	2389	1834	1581	74.8	<0.001	<0.001	<0.001	D × A, D × S, A × S, D × A × S
18:3 <i>n</i> -3	90.8 ^d	245 ^b	1508 ^a	129 ^c	292	695	527	459	33.2	<0.001	<0.001	<0.001	D × A, D × S, A × S, D × A × S
20:5 <i>n</i> -3	–	–	–	417	243	591	450	384	18.0	–	<0.001	<0.001	–
22:6 <i>n</i> -3	–	–	–	307.5	179	436	331	283	13.2	–	<0.001	<0.001	–
Apparent digestibility (%)													
18:2 <i>n</i> -6	69.0 ^b	85.0 ^a	73.4 ^b	73.0 ^b	73.0	77.0	75.4	74.6	4.46	<0.001	0.003	0.551	D × A, D × S
18:3 <i>n</i> -3	62.0 ^c	87.0 ^a	91.0 ^a	77.0 ^b	77.5	81.0	80.0	78.0	4.56	<0.001	0.016	0.361	D × S
20:5 <i>n</i> -3	–	–	–	93.5	90.8	96.2	92.0	95.0	3.95	–	0.047	0.225	–
22:6 <i>n</i> -3	–	–	–	92.8	89.8	95.8	91.3	94.3	3.92	–	0.029	0.222	–
Total appearance/disappearance (μmol/g per d)													
18:2 <i>n</i> -6	–1.17 ^b	–2.09 ^a	–0.59 ^b	–1.01 ^b	–1.92	–0.51	–1.29	–1.14	0.58	<0.001	<0.001	0.395	D × A
18:3 <i>n</i> -6	0.028 ^b	0.031 ^b	0.047 ^a	0.002 ^c	0.029	0.024	0.028	0.025	0.008	<0.001	0.039	0.181	D × A, A × S
20:3 <i>n</i> -6	0.02 ^b	0.03 ^a	0.02 ^b	0.01 ^c	0.03	0.02	0.03	0.03	0.003	<0.001	<0.001	0.929	D × A
20:4 <i>n</i> -6	0.13 ^b	0.20 ^a	0.09 ^c	0.02 ^d	0.13	0.09	0.11	0.11	0.02	<0.001	<0.001	0.982	D × A
18:3 <i>n</i> -3	–0.08 ^b	–0.30 ^b	–1.81 ^a	–0.17 ^b	–0.82	–0.36	–0.58	–0.60	0.25	<0.001	<0.001	0.793	D × A
18:4 <i>n</i> -3	–0.003 ^c	0.007 ^c	0.043 ^b	–0.156 ^a	–0.037	–0.017	–0.030	–0.024	0.012	<0.001	<0.001	0.094	D × A
20:5 <i>n</i> -3	0.01 ^c	0.01 ^c	0.10 ^a	–0.75 ^b	–0.20	–0.11	–0.16	–0.15	0.05	<0.001	<0.001	0.488	D × A
22:5 <i>n</i> -3	0.01 ^c	0.03 ^b	0.09 ^a	0.09 ^a	0.06	0.05	0.05	0.05	0.01	<0.001	0.519	0.857	D × A
22:6 <i>n</i> -3	0.01 ^c	0.02 ^{c,b}	0.05 ^b	–0.46 ^a	–0.12	–0.07	–0.09	–0.09	0.03	<0.001	<0.001	0.366	D × A
Accumulation (% of net intake)													
18:2 <i>n</i> -6	73.1 ^b	74.7 ^b	85.2 ^a	73.0 ^b	67.2	85.8	74.8	78.2	8.80	0.004	<0.001	0.195	–
18:3 <i>n</i> -3	63.9	64.3	65.3	58.4	53.8	72.2	60.8	65.2	9.87	0.329	<0.001	0.134	–
Bioconversion (% of net intake)													
18:2 <i>n</i> -6	4.69 ^a	3.38 ^c	3.88 ^b	0.96 ^d	3.17	3.28	3.24	3.21	0.44	<0.001	0.399	0.852	–
18:3 <i>n</i> -3	9.12 ^a	9.12 ^a	5.80 ^b	0.00 ^c	5.74	6.33	5.42	6.65	1.70	<0.001	0.238	0.018	–
β-Oxidation (% of net intake)													
18:2 <i>n</i> -6	22.1 ^a	21.8 ^a	10.8 ^b	25.9 ^a	29.5	10.8	21.8	18.5	8.85	0.001	<0.001	0.201	–
18:3 <i>n</i> -3	26.7 ^a	26.5 ^a	28.8 ^a	41.5 ^b	40.3	21.4	33.7	28.1	9.37	0.001	<0.001	0.046	–
20:5 <i>n</i> -3	–	–	–	0.66	0.87	0.45	0.68	0.65	0.12	–	<0.001	0.671	–
22:6 <i>n</i> -3	–	–	–	0.46	0.59	0.34	0.48	0.45	0.06	–	<0.001	0.550	–

Palm, palm fat diet; Soya, soyabean oil diet; Lin, linseed oil diet; Fish, fish oil diet; RMSE, root mean squares error; D, diet; A, age; S, sex.

^{a,b,c,d} Mean values within a row for diets with unlike superscript letters were significantly different (*P* < 0.05).

* Significant interaction terms at *P* < 0.05.

Results

In Table 3, the *n*-3 and *n*-6 PUFA content of WB broiler chicken is given. Feeding the Soya diet resulted in higher contents of 18:2*n*-6, 20:3*n*-6 and 20:4*n*-6 compared with the three other diets ($P < 0.001$), whereas the Lin diet induced the highest 18:3*n*-6 and 18:3*n*-3 contents compared with the other diets ($P < 0.001$). The 18:3*n*-3 content in Lin fed birds was 11-fold greater than the average of the other dietary treatments ($P < 0.001$). Birds fed the Fish diet recorded a substantially higher *n*-3 LCPUFA content (20:5*n*-3, 22:5*n*-3 and 22:6*n*-3) compared with the other diets ($P < 0.001$). The 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 content in Fish fed birds was 14.3-, 8.4- and 5-fold greater compared with the average of the non-Fish fed birds. There was a trend for an effect of diet on the total body fatty acid content ($P = 0.051$), with lower values for the Palm and Fish diets compared with the Soya and Lin diets. As expected, age and sex had a significant effect on the total body fatty acid content.

PUFA intake, apparent digestibility, total appearance/disappearance and fate of 18:2*n*-6 and 18:3*n*-3 are presented in Table 4. Apparent digestibility for 18:2*n*-6 was significantly higher for Soya fed birds compared with the other diets ($P < 0.001$). Apparent digestibility of 18:3*n*-3 was higher on the Soya and Lin diets than on the Palm and Fish diets ($P < 0.001$).

Disappearance of PUFA may imply elongation and desaturation to longer chain metabolites or utilisation of their carbon skeleton through β -oxidation for energy production. Disappearance of 18:2*n*-6 and appearance of 20:4*n*-6 were higher in Soya fed birds in contrast with the other dietary treatments ($P < 0.001$). Disappearance of 18:3*n*-3 and appearance of 18:4*n*-3 and 20:5*n*-3 were higher in Lin fed birds compared with the other diets ($P < 0.001$). Appearance of 22:5*n*-3 was similar for the Lin and Fish fed birds and higher than for the Palm and Soya diets ($P < 0.001$). Appearance of 22:6*n*-3 was higher on the Lin diet than on the Soya and Palm diets ($P < 0.05$ only for difference Lin *v.* Palm diet). Birds of the Fish group recorded a net disappearance of 18:4*n*-3, 20:5*n*-3 and 22:6*n*-3 ($P < 0.001$).

Birds fed the Lin diet had a higher 18:2*n*-6 accumulation (% of net intake) compared with the other diets ($P < 0.01$). Bioconversion (% of net intake) of 18:2*n*-6 to longer chain/more unsaturated fatty acids decreased in the order Palm > Lin > Soya > Fish ($P < 0.001$). The 18:2*n*-6 β -oxidation (% net intake) on the Lin diet was 2-fold lower than on the other diets ($P < 0.001$). Accumulation of 18:3*n*-3 was not significantly different among the diets. Birds fed the Fish diet did not demonstrate bioconversion of 18:3*n*-3 to 18:4*n*-3. However, 18:3*n*-3 bioconversion on the Palm and Soya diets was similar and 1.5-fold higher than on the Lin diet ($P < 0.001$). The 18:3*n*-3 β -oxidation on the Fish diet was 1.4-fold higher than on the other dietary treatments ($P < 0.001$).

The effect of age was significant for PUFA intake, apparent digestibility, appearance/disappearance (except of 22:5*n*-3) and the proportions of the net intake of 18:2*n*-6 and 18:3*n*-3 accumulated and β -oxidised. Birds slaughtered at day 42 of age, when compared with the 7–14 d age period, had higher values for PUFA intake, PUFA apparent digestibility and 18:2*n*-6 and 18:3*n*-3 accumulation, but lower values for PUFA appearance/disappearance and β -oxidation. The effect

Table 5. Accretion of Δ -5 and Δ -6 desaturated and elongated fatty acids in broilers

	Diet (n 12)				Age (n 24)		Sex (n 24)		P				
	Palm	Soya	Lin	Fish	7–21 d	21–42 d	Male	Female	RMSE	Diet	Age	Sex	Interactions*
Accretion of Δ -5 desaturated fatty acid ($\mu\text{mol/g per d}$)													
20:3 <i>n</i> -6 \rightarrow 20:4 <i>n</i> -6	0.13 ^b	0.20 ^a	0.09 ^c	0.02 ^d	0.13	0.09	0.11	0.11	0.02	<0.001	<0.001	0.982	D \times A
20:4 <i>n</i> -3 \rightarrow 20:5 <i>n</i> -3	0.02 ^c	0.06 ^b	0.25 ^a	0.00 ^d	0.10	0.07	0.08	0.09	0.01	<0.001	<0.001	0.060	D \times A, A \times S, D \times A \times S
Accretion of Δ -6 desaturated fatty acid ($\mu\text{mol/g per d}$)													
18:2 <i>n</i> -6 \rightarrow 18:3 <i>n</i> -6	0.19 ^b	0.27 ^a	0.16 ^b	0.03 ^c	0.19	0.14	0.16	0.16	0.02	<0.001	<0.001	0.728	D \times A
18:3 <i>n</i> -3 \rightarrow 18:4 <i>n</i> -3	0.02 ^c	0.07 ^b	0.30 ^a	0.00 ^d	0.11	0.08	0.09	0.10	0.01	<0.001	<0.001	0.094	D \times A
24:5 <i>n</i> -3 \rightarrow 24:6 <i>n</i> -3	0.007 ^c	0.023 ^b	0.051 ^a	0.000 ^d	0.024	0.018	0.020	0.022	0.005	<0.001	<0.001	0.062	D \times A, A \times S, D \times A \times S
Accretion of elongated fatty acid ($\mu\text{mol/g per d}$)													
18:3 <i>n</i> -6 \rightarrow 20:3 <i>n</i> -6	0.16 ^b	0.23 ^a	0.11 ^c	0.03 ^d	0.16	0.11	0.14	0.14	0.02	<0.001	<0.001	0.974	D \times A
18:4 <i>n</i> -3 \rightarrow 20:4 <i>n</i> -3	0.02 ^c	0.06 ^b	0.25 ^a	0.00 ^d	0.10	0.07	0.08	0.09	0.01	<0.001	<0.001	0.060	D \times A, A \times S, D \times A \times S
20:5 <i>n</i> -3 \rightarrow 22:5 <i>n</i> -3	0.02 ^c	0.05 ^b	0.15 ^a	0.09 ^d	0.08	0.07	0.07	0.08	0.01	<0.001	<0.062	0.453	D \times A
22:5 <i>n</i> -3 \rightarrow 24:5 <i>n</i> -3	0.01 ^c	0.02 ^b	0.05 ^a	0.00 ^d	0.02	0.01	0.02	0.02	0.01	<0.001	<0.001	0.062	D \times A, A \times S, D \times A \times S

Palm, palm fat diet; Soya, soyabean oil diet; Lin, linseed oil diet; Fish, fish oil diet; RMSE, root mean squares error; D, diet; A, age; S, sex.

^{a,b,c,d} Mean values within a row for diets with unlike superscript letters were significantly different ($P < 0.05$).

* Significant interaction terms at $P < 0.05$.

of sex on the above-mentioned variables was significant for 18:2*n*-6 and 18:3*n*-3 intake, 18:3*n*-3 bioconversion and 18:3*n*-3 β -oxidation. Males had a greater 18:2*n*-6 and 18:3*n*-3 intake and 18:3*n*-3 β -oxidation ($P < 0.05$), whereas females showed a higher bioconversion of 18:3*n*-3 to 18:4*n*-3 ($P < 0.05$).

Accretion of Δ -5 and Δ -6 desaturated fatty acids and accretion of elongated fatty acids are presented in Table 5. Conversion of 20:3*n*-6 to 20:4*n*-6 as an indication of apparent Δ -5 desaturation activity and conversion of 18:2*n*-6 to 18:3*n*-6 as an indication of apparent Δ -6 desaturation activity in the *n*-6 pathway were higher in Soya fed birds compared with the other dietary treatments ($P < 0.001$). The Δ -5 desaturation of 20:4*n*-3 to 20:5*n*-3 was higher in Lin fed birds compared with the other diets ($P < 0.001$). Similarly, Δ -6 desaturation of 18:3*n*-3 to 18:4*n*-3 and 22:5*n*-3 to 22:6*n*-3 was greater on the Lin diet compared with the other diets ($P < 0.001$). Elongation of 18:3*n*-6 towards 20:3*n*-6 was higher in Soya fed birds than in the other diets ($P < 0.001$), and elongation of 18:4*n*-3, 20:5*n*-3 and 22:5*n*-3 was higher in birds fed the Lin diet than in the other diets ($P < 0.001$). Apparent desaturation activity was higher at 7–21 d of age than at 21–42 d of age ($P < 0.001$). Apparent elongation activity on 18:3*n*-6, 18:4*n*-3 and 22:5*n*-3 was higher in birds slaughtered on day 21 compared with day 42 ($P < 0.001$), whereas elongation of 20:5*n*-3 to 22:5*n*-3 was not affected by age ($P > 0.05$). Sex had no influence on the above-mentioned variables ($P > 0.05$).

The apparent Δ -6 desaturase activity acting on 18:3*n*-3 and 18:2*n*-6 was plotted against their respective net intake both expressed as $\mu\text{mol/g per d}$ (Figs 1 and 2). The apparent Δ -6 desaturase activity on 18:3*n*-3 showed a strong positive linear relationship with the 18:3*n*-3 net intake (R^2 0.98), indicating that the higher the dietary 18:3*n*-3 supply, the higher the resultant product (18:4*n*-3). The response of apparent Δ -6 desaturase activity to 18:2*n*-6 net intake was positive but curvilinear (R^2 0.66), showing that by enhancing substrate (18:2*n*-6) availability, the efficiency of Δ -6 desaturase (18:3*n*-6 production) increased only up to a certain level. The Δ -5 desaturase activity on 20:4*n*-3 manifested a strong positive relationship (R^2 0.99) with the summed 18:4*n*-3 and 18:3*n*-3 dietary supply (Fig. 3).

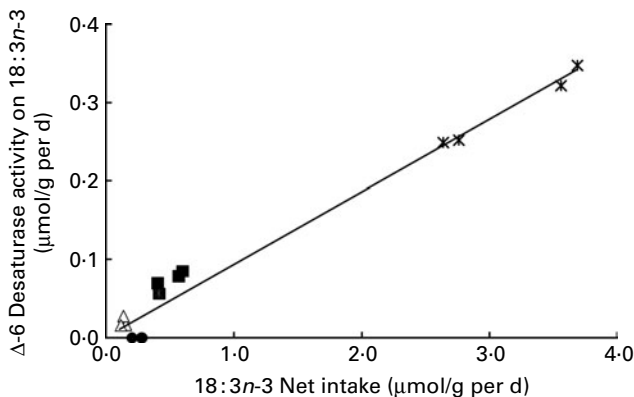


Fig. 1. Apparent Δ -6 desaturase activity on 18:3*n*-3 in relation to the 18:3*n*-3 net intake ($\mu\text{mol/g per d}$) across four diets, age and sex. Linear regression equation: $Y = 0.0030 + 0.0919X$; R^2 0.98; root mean squares error = 0.019 (n 16). ●, Fish oil diet; ×, linseed oil diet; ■, soyabean oil diet; Δ, palm fat diet.

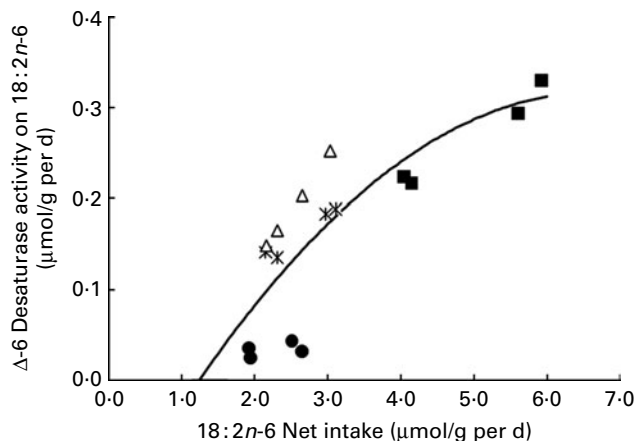


Fig. 2. Apparent Δ -6 desaturase activity on 18:2*n*-6 in relation to the 18:2*n*-6 net intake ($\mu\text{mol/g per d}$) across four diets, age and sex. Broken-line quadratic regression equation: $Y = 0.3167$ if $X > 6.669$ and $Y = 0.3167 - 0.0107 \times (6.669 - X)^2$ if $X \leq 6.669$; R^2 0.66; root mean squares error = 0.058 (n 16). ●, Fish oil diet; ×, linseed oil diet; ■, soyabean oil diet; Δ, palm fat diet.

Significant interaction terms are mentioned in the tables, but these effects are not further discussed here since they were negligible compared with the main effects and did not compromise the main conclusions. Most of the significant interaction effects were diet \times age effects. P -values for the interaction effects were considerably smaller compared with the main effects. In addition, the significant interaction effects were the result of scale differences in most cases, i.e. the difference between the two age groups differed among diets in magnitude but not in sign (and vice versa) in case of a significant diet \times age effect. In few cases, the effect of age was opposite or absent in one diet versus the other diets, mostly when very low values occurred for a diet. The only marked diet \times age interaction effect was for the digestibility of 18:2*n*-6 and 18:3*n*-3, which was approximately 10% lower in the young compared with the older birds on the Palm diet, whereas the age effect was negligible on the other diets.

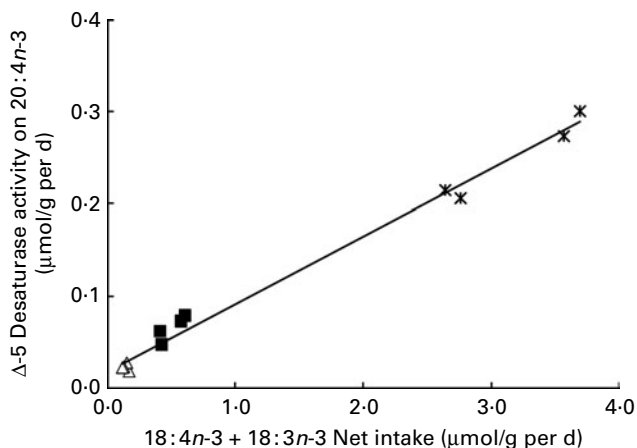


Fig. 3. Apparent Δ -5 desaturase activity on 20:4*n*-3 in relation to the 18:4*n*-3 + 18:3*n*-3 net intake ($\mu\text{mol/g per d}$) across three diets, age and sex. Linear regression equation: $Y = 0.0197 + 0.0728X$; R^2 0.99; root mean squares error = 0.011 (n 12). ×, linseed oil diet; ■, soyabean oil diet; Δ, palm fat diet.

Discussion

The effect of diet and age on the fatty acid apparent digestibility in the present study is in agreement with previous studies in broiler chickens^(15–17). Many of the physiological functions necessary for lipid digestion are immature at hatch and develop over the next weeks. Bile salt secretion appears to be the first limiting factor for lipid digestion through the first few weeks post hatch⁽¹⁵⁾. In the present experiment, the apparent digestibility values of 18:2n-6, 18:3n-3 and 20:5n-3 are in the same range as recently reported specifically for broiler chickens⁽¹⁷⁾. An earlier study described higher animal fat digestibility in female compared with male chickens⁽¹⁵⁾. A slightly higher PUFA digestibility in female birds fed the Fish diet was observed in the present study; however, this difference was not statistically different. It should also be kept in mind that the digestibilities in the present experiment may be overestimated due to PUFA microbial biohydrogenation in the hindgut⁽¹⁷⁾.

The ingested PUFA face one of the three fates in the body, i.e. accumulation, conversion to longer chain/more unsaturated metabolites or β -oxidation. The WB fatty acid balance method allows quantifying the fate of the WB 18:2n-6 and 18:3n-3 relative to the net intake. Irrespective of the dietary treatment, broiler chickens seem to have a preference for accumulation of 18:2n-6 above 18:3n-3. Reviewing the literature, quantitative data on fatty acid metabolism in broiler chicken are scarce. Accumulation of 18:2n-6 and 18:3n-3 in WB level of growing pig fed a maize–soyabean meal basal diet (without added fat) was approximately 67 and 48% of digestible fatty acid, respectively⁽¹⁸⁾. In a WB fatty acid study with Murray cod fish fed with Lin, 40 and 53% accumulation of net intake for 18:2n-6 and 18:3n-3 were respectively reported⁽¹⁹⁾. These values are lower than for the 18:2n-6 and 18:3n-3% net intake accumulation in Lin fed broilers in the present study. In a recent study with rainbow trout fed with fish oil, 91 and 60% net intake of 18:2n-6 and 18:3n-3 were respectively deposited in the WB⁽¹⁴⁾. However, in Lin fed trout, the corresponding values were 70 and 58% of net intake for 18:2n-6 and 18:3n-3, respectively. Therefore, it is possible to speculate that comparing chicken with rainbow trout both fed with fish oil, 18:2n-6 and 18:3n-3 accumulation is greater in fish than in chicken. Consequently, 18:2n-6 and 18:3n-3 accumulation appears greater in chicken compared with fish when fed Lin.

Across the four dietary treatments, the conversion rate of 18:3n-3 to 18:4n-3 was 1.8-fold higher than the conversion rate of 18:2n-6 to 18:3n-6 indicating a higher affinity of Δ -6 desaturase for 18:3n-3 than for 18:2n-6. A relatively lower 18:3n-3 bioconversion and increased β -oxidation rate in the Lin diet in contrast with Palm and Soya fed birds are in agreement with a previous report by Vermunt *et al.*⁽²⁰⁾, stating that diets rich in 18:3n-3 increase 18:3n-3 β -oxidation, coincident with decreasing its conversion rate to longer and more unsaturated n-3 LCPUFA. In a WB fatty acid study with Murray cod fish fed Lin diet, 4 and 8% of 18:2n-6 and 18:3n-3 net intake were respectively converted to longer chain metabolites⁽¹⁹⁾. Hence, it seems there is a larger 18:3n-3 conversion in Lin fed Murray cod fish than in Lin fed chicken in our experiment. Similar to our findings, rainbow trout fed with fish oil did not demonstrate conversion

of 18:3n-3 to 18:4n-3 in WB⁽¹⁴⁾. Feeding vegetable oil rather than fish oil increases desaturase and elongase activity as well as upregulates liver fatty acid elongase and FAD5 gene expression^(14,21). This explains the higher conversion of 18:3n-3 to 18:4n-3 in the Palm, Soya and Lin diets (5- to 9-fold) compared with the Fish diet in the present study. The 18:2n-6 bioconversion in rainbow trout fed with fish oil⁽¹⁴⁾ was 1.8-fold higher than in chicken fed fish oil in the present study. Comparing rainbow trout fish fed with Lin with the Lin treatment in our experiment indicated that the 18:2n-6 conversion was 1.2-fold higher in chicken albeit the 18:3n-3 conversion was 2-fold higher in fish.

The higher 18:3n-3 bioconversion in female birds of the present study is in agreement with previous findings in other species, e.g. human and rat^(22,23). Both the role of steroid hormones and other sex differences in metabolism may be involved. The n-3 LCPUFA content in plasma and tissues of rats appear to be positively related to circulating concentrations of oestradiol and progesterone and negatively associated with circulating concentrations of testosterone⁽²⁴⁾, and testosterone administration to rats strongly depressed Δ -5 and Δ -6 desaturase activity⁽²²⁾. In addition, fat β -oxidation was found to be greater in men than in women in several studies^(23,25), corresponding to our finding that β -oxidation of 18:2n-6 and 18:3n-3 was higher in males than in females. Therefore, the availability of 18:3n-3 for conversion to metabolites may be higher in females than in males. However, caution is needed when comparing the present results with those from studies involving mature animals or human subjects, since the male chickens in the present study were still immature. It is thus not clear whether the observed differences are mainly due to an effect of sex hormones or due to differences in energy metabolism.

The WB fatty acid balance method applied in the present study provided an estimation of apparent *in vivo* Δ -5 and Δ -6 desaturase activity. Regression analysis demonstrated a linear response in Δ -6 desaturase activity in the n-3 pathway to an increasing supply of the substrate 18:3n-3. The same trend was observed for Δ -5 desaturase activity. On the other hand, the response in Δ -6 desaturase activity in the n-6 pathway to an increasing supply of 18:2n-6 was curvilinear. However, it should be noted that the range of net intake of 18:2n-6 was larger than for 18:3n-3, and that the curvilinear response could be partly due to the considerably lower Δ -6 desaturase activity on the Fish diet compared with a similar 18:2n-6 net intake values on the other diets.

Crespo & Esteve-Garcia⁽¹⁶⁾ reported that the total β -oxidation of n-6 PUFA was 29 and 11% of net intake, and 29 and 14% for n-3 PUFA, in linseed and sunflower oil fed broiler chickens, respectively. However, the individual fates of 18:2n-6 and 18:3n-3 were not reported. In growing pigs fed a maize–soyabean meal diet, 30% of the digested Σ n-6 fatty acid and 52% of the digested 18:3n-3 were β -oxidised at WB level⁽¹⁸⁾. The 18:3n-3 β -oxidation reported in the present study was 31% (across diets, age and sex), and therefore it seems to be lower in broiler chickens in contrast with swine. In rat, Leyton *et al.*⁽²⁶⁾ measured exhalation of ¹⁴CO₂ and demonstrated that the β -oxidation rate of 18:2n-6 and 18:3n-3 was 48 and 64%, respectively. In human, over 30% of labelled 18:3n-3 administered were used as energy source in man; however, lower values (22%) were reported

in women^(23,27). Consistently, numerically lower β -oxidation values for 18:2*n*-6 and 18:3*n*-3 were also recorded in female chickens in the present study.

In fish, the β -oxidation of 18:2*n*-6 and 18:3*n*-3 was 29 and 8% of net intake, respectively, at WB level of Murray cod fish fed with Lin⁽¹⁹⁾; while in rainbow trout fed with fish oil, 18:2*n*-6 and 18:3*n*-3 β -oxidation was reported as 7 and 40% of net intake⁽¹⁴⁾. Comparing our findings with the latter study, which was implemented with similar methodologies, reveals that 18:2*n*-6 β -oxidation in fish oil fed chicken is about 3- to 4-fold higher than in rainbow trout, while 18:3*n*-3 β -oxidation is approximately similar in the two species (40 v. 41% of net intake). In Lin fed rainbow trout, the β -oxidation of 18:2*n*-6 and 18:3*n*-3 was 26 and 29% of the net intake⁽¹⁴⁾. This suggests that 18:2*n*-6 β -oxidation in linseed fed chicken is 3-fold lower than in linseed fed rainbow trout. On the other hand, 18:3*n*-3 β -oxidation in linseed fed chicken from the present study corresponds with linseed fed rainbow trout fish (29% net intake). It is at this point important to highlight some fundamental differences between fish and birds basic nutrition: fish are cold-blooded animals and have minor metabolic energy requirements (i.e. no energetic costs for maintaining the position and minor energetic costs for the detoxification of N containing compounds). Birds derive the majority of their energy requirements from dietary carbohydrates; while in fish, carbohydrates are very poorly utilised. Accordingly, the lipid content of diets for the two groups of animals is remarkably different (approximately 8% in broiler chickens and approximately 25% in trout). Despite these important differences, our comparisons depicted earlier are relative to the fate of specific fatty acids as percentage of net intake, and therefore these differences have to be considered levelled. Thus, these comparisons are sound and useful in providing a better understanding of PUFA metabolism in different farmed animals.

The stage of the growth and development influences fatty acid β -oxidation⁽²⁸⁾. In our experiment, β -oxidation of 18:2*n*-6, 18:3*n*-3, 18:4*n*-3, 20:5*n*-3 and 22:6*n*-3 was higher in 21 d-old birds compared with 42 d-old birds. This corresponds with the fact that young fast growing chickens have a higher metabolism rate compared with the same birds approaching slaughter age.

The WB fatty acid balance method provides information about the overall fatty acid metabolism in the body. The method is comparatively inexpensive, easy and feasible in animal research laboratories⁽⁷⁾. However, there are some assumptions and limitations in this approach that should be considered. The method does not allow to study LCPUFA retroconversion. Likewise, fatty acid β -oxidation is estimated based on the fatty acid disappearance, while the use of fatty acids for the synthesis of non-fatty acid metabolites, e.g. hormones or PG, is not considered (though quantitatively extremely limited). However, it is important to underline that other methods employing labelled fatty acid techniques are accomplished with similar assumptions. The main difference between the WB method and other methods used for fatty acid metabolism studies (e.g. perfused isolated liver isotopical study) is the time frame^(14,29). In fact, the WB method needs a longer time frame compared with the other methods. Consequently, the average enzyme activity

(enzyme velocity relative to total enzyme product over time) is estimated in a longer time frame. Therefore, estimated apparent enzyme activity may deviate from the instantaneous enzyme activity measured at a given time, but it likely provides useful information relative to whole metabolism in growing animals, resulting in a reliable estimation of the apparent *in vivo* metabolic activity.

In consideration of the fact that *n*-3 LCPUFA (namely 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3) are reportedly beneficial to human health^(1,3,5), there is great interest towards finding possible ways to increase the *n*-3 LCPUFA content of farmed animals. In the present study, we recorded that in broiler chickens fed the Lin diet for 42 d, hence receiving the highest amount of 18:3*n*-3, a total of 5.8% of the net intake of 18:3*n*-3 was eventually converted to *n*-3 LCPUFA. On a molar basis, the conversion of 18:3*n*-3 to 20:5*n*-3 and 22:6*n*-3 was 2.7 and 3.3% on the Palm diet, 1.6 and 2.7% on the Soya diet and 2.0 and 1.2% on the Lin diet, respectively. In rainbow trout fed a Lin-based diet, it was reported that 8.8% of the net 18:3*n*-3 dietary intake was bioconverted to *n*-3 LCPUFA⁽¹⁴⁾. Thus, in comparison to the values reported in human subjects^(4,5), the 18:3*n*-3 to *n*-3 LCPUFA bioconversion capacity of broiler chicken and rainbow trout appears to be remarkably greater, particularly the synthesis of 22:6*n*-3. Two important conclusions can be obtained from this observation. Generalising, it can be speculated that the efficiency of the bioconversion of 18:3*n*-3 to *n*-3 LCPUFA seems to be inversely related to evolution (rainbow trout > broiler chicken > human). Secondly, the inclusion of Lin (or other sources of 18:3*n*-3) in animal feed results in beneficial net production of *n*-3 LCPUFA. Considering breast and thigh meat (without skin) as the only edible parts of the chicken carcass, and using their proportion of the body weight and their fatty acid composition⁽⁹⁾, it was calculated that 100 g of meat (breast and thigh) provided 62 mg *n*-3 LCPUFA (20:5*n*-3, 22:5*n*-3 and 22:6*n*-3) on the Lin diet. On the other hand, the actual total intake of 18:3*n*-3 to produce the live biomass corresponding to 100 g of edible meat was 7581 mg. On the Fish diet, 100 g meat provided 198 mg *n*-3 LCPUFA, corresponding to an intake by the broiler chickens directly of 4284 mg *n*-3 LCPUFA. Although the supply of *n*-3 LCPUFA was 3-fold higher in meat from Fish fed chicken compared with Lin fed chicken, the latter meat did not require consumption of *n*-3 LCPUFA by the broiler chicken. Thus, from a human nutrition viewpoint, the present data suggest that it is more beneficial to consume chicken fed Lin than the practice of direct consumption of Lin or other sources of 18:3*n*-3 alone by human subjects, in consideration that the conversion efficiency of 18:3*n*-3 to *n*-3 LCPUFA in human subjects is lower than 0.80%⁽³⁰⁾. Additionally, from an environmental viewpoint, the use of fish oil in chicken nutrition seems to be a relatively wasteful practice of this precious and limited natural resource.

In conclusion, the metabolism (elongation, desaturation and β -oxidation) of PUFA in broiler chickens is mainly affected by the dietary fatty acid source rather than the animal factors. Dietary provision of 18:3*n*-3 promotes the apparent elongation and desaturation activity on fatty acids in the *n*-3 pathway. Hence, 18:3*n*-3 bioconversion relative to the net intake of this fatty acid seems to be limited by the dietary supply. Feeding fish oil suppresses apparent elongase and

desaturase activity, but it was responsible for significantly higher total deposition of n-3 LCPUFA. Apparent elongase and desaturase activity appeared to be higher in young broiler chickens (21 d) compared with those at conventional slaughter age (42 d). Sex has only a marginal effect on the PUFA metabolism in broiler chicken.

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