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Abstract

The effects of dietary saturated fatty acids and polyunsaturated fatty acids (PUFA) of the *n*-3 and *n*-6 series on weight gain, body composition and substrate oxidation were investigated in broiler chickens. At 3 weeks of age three groups of chickens (*n* 30; ten birds per group) were fed the fat-enriched experimental diets for 5 weeks. These diets were isonitrogenous, isoenergetic and contained 208 g protein/kg and 80 g edible tallow, fish oil or sunflower oil/kg; the dietary fatty acid profiles were thus dominated by saturated fatty acids, *n*-3 PUFA or *n*-6 PUFA respectively. Resting RQ was measured in five birds from each treatment group during weeks 4 and 5 of the experiment. There were no significant differences between treatments in total feed intake or final body mass. Birds fed the PUFA diets had lower RQ and significantly reduced abdominal fat pad weights ($P<0{\cdot}01$) compared with those fed tallow. The dietary lipid profile changes resulted in significantly greater partitioning of energy into lean tissue than into fat tissue (calculated as breast lean tissue weight:abdominal fat mass) in the PUFA groups compared with the saturated fat group ($P<0{\cdot}01$; with no difference between the *n*-3 and *n*-6 PUFA groups). In addition, the PUFA-rich diets lowered plasma concentrations of serum triacylglycerols and cholesterol. The findings indicate that dietary fatty acid profile influences nutrient partitioning in broiler chickens.

Keywords

metabolism, avian, alter, dietary, acids, 6, 3, n, fat, abdominal, deposition, fatty

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Dietary *n*-3 and *n*-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition

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The effects of dietary saturated fatty acids and polyunsaturated fatty acids (PUFA) of the *n*-3 and *n*-6 series on weight gain, body composition and substrate oxidation were investigated in broiler chickens. At 3 weeks of age three groups of chickens (*n* 30; ten birds per group) were fed the fat-enriched experimental diets for 5 weeks. These diets were isonitrogenous, isoenergetic and contained 208 g protein/kg and 80 g edible tallow, fish oil or sunflower oil/kg; the dietary fatty acid profiles were thus dominated by saturated fatty acids, *n*-3 PUFA or *n*-6 PUFA respectively. Resting RQ was measured in five birds from each treatment group during weeks 4 and 5 of the experiment. There were no significant differences between treatments in total feed intake or final body mass. Birds fed the PUFA diets had lower RQ and significantly reduced abdominal fat pad weights ($P<0{\cdot}01$) compared with those fed tallow. The dietary lipid profile changes resulted in significantly greater partitioning of energy into lean tissue than into fat tissue (calculated as breast lean tissue weight:abdominal fat mass) in the PUFA groups compared with the saturated fat group ($P<0{\cdot}01$; with no difference between the *n*-3 and *n*-6 PUFA groups). In addition, the PUFA-rich diets lowered plasma concentrations of serum triacylglycerols and cholesterol. The findings indicate that dietary fatty acid profile influences nutrient partitioning in broiler chickens.

Polyunsaturated fatty acids: Chickens: Triacylglycerols: Energy metabolism: Body composition

Nutritional studies in man and rats have shown that energy balance and body fat content can be manipulated by altering dietary polyunsaturated fatty acids (PUFA):saturated fatty acids (P:S), specifically by the inclusion in the diet of the long-chain PUFA (Field *et al.* 1990; Pan *et al.* 1994; Luo *et al.* 1996; Couet *et al.* 1997). An increase in the dietary P:S by the inclusion of *n*-3 PUFA has direct effects on glucose and lipid metabolism and decreases body fat mass. For example, rats fed on a diet containing 30 % (w/w) fish oil had reduced circulating insulin concentrations and improved insulin-stimulated glucose uptake compared with those on diets containing equal amounts of vegetable or animal oils (Storlien *et al.* 1991; Luo *et al.* 1996). Furthermore, rats on the fish oil diet also showed reduced epididymal fat pad mass without any change in body weight. Similar metabolic and body composition changes in response to the dietary inclusion of *n*-3 PUFA have been reported for man. When a diet enriched with fish oil was fed to young adult men and women, basal blood glucose concentrations increased, basal insulin concentrations decreased, and there was a significant reduction ($P<0{\cdot}05$) in overall body fat mass (Couet *et al.* 1997). Associated with reduced fat mass, these subjects also had an increased resting metabolic rate (PMR) and a decreased basal RQ, indicating increased reliance on lipid oxidation.

Birds generally have a high capacity for lipid biosynthesis (Pearce, 1980; Annison, 1983; Klasing, 1998); including the modern broiler or meat chicken, which has the propensity to become excessively fat. This accumulation

Abbreviations: DHA, docosahexaenoic acid (22 : 6); EPA, eicosapentaenoic acid (20 : 5); P:S, polyunsaturated:saturated fatty acids; PUFA, polyunsaturated fatty acid; RMR, resting metabolic rate.

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of fat has significant health implications, as carcass fat of these birds is an important source of dietary fat for man. To reduce this problem, research examining the possibility of selecting chickens with a reduced tendency to accumulate triacylglycerol (Griffin, 1993), as well as studies identifying to what extent the composition of carcass triacylglycerol can be modified by changes in dietary lipid intake (Leskanich & Noble, 1997), has been conducted. The aim of many of these studies has been to enhance the human dietary intake of PUFA. Many studies have manipulated the *n*-3 and *n*-6 PUFA content of carcass triacylglycerols (Leskanich & Noble, 1997), but few (Sanz *et al.* 2000) have examined the effects of PUFA on avian metabolism. The present study examines both morphological and physiological responses of broiler chickens to different sources of dietary fatty acids: fish oil (*n*-3 PUFA); sunflower oil (*n*-6 PUFA); edible tallow.

Materials and methods

Birds, diets and study design

Thirty-five mixed-gender broiler chicks (Inghams strain TM70, Inghams Enterprises Pty Ltd., Liverpool, NSW, Australia), obtained from a commercial hatchery, were placed in a temperature-controlled brooder with raised wire floors and continuous fluorescent lighting. Chicks were given free access to commercial chick starter crumbles (230 g crude protein (N × 6·25) kg diet) and water for a period of 3 weeks. At the end of this period, thirty chicks were randomly divided into three groups (*n* 10) and placed in individual cages with free access to water and fed the experimental diets for 5 weeks. The diets contained 80 g edible-grade tallow (containing less than 1 % (w/w) free fatty acids), sunflower oil or fish oil/kg (Table 1). The fatty acid composition of the three experimental diets is shown in Table 2.

The fish oil (*n*-3 PUFA) and sunflower oil (*n*-6 PUFA) diets contained significantly less ($P<0·01$) saturated (palmitic 16 : 0; stearic 18 : 0) and monounsaturated (oleic 18 : 1 *cis* 9) fatty acids than the tallow diet. In contrast, the sunflower oil diet contained a significantly higher ($P<0·01$) proportion of linoleic acid (18 : 2) when compared with either the fish oil or the tallow diets. The fish oil diet contained the *n*-3 fatty acids eicosapentaenoic acid (20 : 5; EPA) and docosahexaenoic acid (22 : 6; DHA), which were not detected in either the sunflower oil or the tallow diets. The saturated fatty acids:monounsaturated fatty acids:PUFA mass was 31 : 40 : 26 for the tallow diet, 28 : 25 : 43 for the fish oil diet and 13 : 23 : 64 for the sunflower oil diet.

The diets were prepared weekly. Feed ingredients and diets were kept at room temperature, with the exception of fish oil which was maintained at 4°C. The fish oil was stabilised with an antioxidant by the manufacturer and the vitamin–mineral premix contained ethoxyquin. Feed in cages was replenished every 2–3 d following the collection of feed residues from the previous period. Estimates of weekly feed intake were made by subtracting the total weekly residue weight from the total weight of feed offered for that week. During weeks 4 and 5 of the experiment the resting RQ and RMR were determined for five birds from each diet group. At the end of week 5 a blood sample (5 ml) was collected by venipuncture from the jugular vein of each bird using heparinized syringes (Terumo, Tokyo, Japan). Blood samples were kept on ice until centrifuged within 1 h of collection, and the separated plasma, was stored at −20 °C until assayed. The abdominal fat pad and breast muscles of each chicken were removed and weighed following cervical dislocation.

Experimental procedures which involved the use of birds were approved by the University of Sydney Animal Care and Ethics Committee and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Table 1. Composition (g/kg) of the experimental diets

	Fish oil	Sunflower oil	Tallow
Ingredient			
Maize	566	566	566
Soyabean meal	318	318	318
Fish oil	80	–	–
Sunflower oil	–	80	–
Tallow	–	–	80
Limestone	11·6	11·6	11·6
Dicalcium phosphate	14·3	14·3	14·3
Salt	3·0	3·0	3·0
DL-Methionine	1·0	1·0	1·0
Vitamin–trace mineral premix*	5·0	5·0	5·0
Choline chloride	2·0	2·0	2·0
Determined composition (g/kg)			
Crude protein (N × 6·25)	202	209	221
Metabolisable energy (MJ/kg)	14·0	14·2	13·7
Total fat (g/kg)	111	107	111

* Supplied (mg/kg diet): *trans*-retinol 3·3, cholecalciferol 87·5 μg, DL-α-tocopheryl acetate 20, menadione 2, thiamine 1·5, riboflavin 8, calcium pantothenate 15, niacin 30, pyridoxine 5, folic acid 2, cyanocobalamin 15 g, biotin 100 μg, Mn 75, Zn 50, Cu 5, Mo 1·6, Co 300 μg, I 1, Fe 20, Se 100 μg, choline chloride 300, ethoxyquin 125.

Table 2. Determined fatty acid composition (g/100 g) of the experimental diets*

Fatty acid	Fish oil	Sunflower oil	Tallow
14 : 0	5·17	ND	1·04
16 : 0	16·03	8·55	25·24
16 : 1	6·03	ND	1·12
18 : 0	3·43	4·31	6·06
18 : 1 *cis* 9	14·07	23·27	38·03
18 : 2*n*-6	19·77	62·73	25·80
18 : 3*n*-3	1·81	1·13	1·49
20 : 0	2·94	ND	ND
20 : 5*n*-3	13·41	ND	ND
22 : 6*n*-3	8·77	ND	ND
Total saturated (S)	27·6	12·9	32·3
Total monounsaturated	24·94	23·27	39·15
Total polyunsaturated (P):*n*-6	19·77	62·73	25·80
n-3	23·59	1·13	1·47
P:S†	1·57	4·97	0·84

ND, not detected.
* For details of diets, see Table 1 and p. 12.
† *n*-6 + *n*-3/14 : 0 + 16 : 0 + 18 : 0 + 20 : 0.

Apparent metabolisable energy

The apparent metabolisable energy values were determined using a classical total excreta collection method (Mollah *et al.* 1983). Gross energy was determined for excreta and diet samples using an automatic adiabatic bomb calorimeter (Gallenkamp and Co. Ltd, London, UK) that had been standardised using benzoic acid (Mollah *et al.* 1983).

Plasma metabolites

Plasma glucose, triacylglycerol, and total cholesterol measurements were determined using a Roche Cobas Mira (Roche, Basel, Switzerland) automatic analyser with commercially-available assay kits (Glucose Unimate 5 Gluc HK; Triglycerides Unimate 5 Trig; and Cholesterol Unimate 5 Chol, Roche Products Pty Ltd, Frenchs Forest, NSW, Australia). Plasma long-chain non-esterified fatty acids were determined by enzymic analysis using reagents and methods supplied by Boehringer Mannheim GmbH, Mannheim, Germany.

Resting RQ *and metabolic rate*

O_2 consumption and CO_2 production rates were determined using open-system respirometry, by measuring O_2 and CO_2 contents of inlet- and outlet- chamber air using Sable Systems FC-1 O_2 and CA-1 CO_2 analysers (Sable Systems Int. Inc, Henderson, Nevada, USA). The chambers consisted of a rectangular plastic box fitted over a standard broiler chicken holding cage (645 × 355 × 615 mm), with a transparent top and front panel to permit illumination and opaque sides to minimise interaction between adjacent birds. Birds were provided with free access to feed and water throughout the measurement period, which took place between 08.00 and 17.00 hours in a continuously-lighted room maintained at 25°C. Air was provided to each chamber at 3·5 litres/min using calibrated rotameters. After a 2 h adjustment period, chambers were selectively sampled for air composition using a Sable Systems Respirometer Multiplexer (V 2.0) (Sable Systems Int. Inc, Henderson, Nevada, USA). Voltage output from the O_2 and CO_2 analysers were recorded at 5 s intervals and each bird was sampled for 11 min/h. Values for O_2 consumption and CO_2 production were corrected to standard temperature and pressure conditions after appropriate adjustment for volume effects associated with RQ different from unity (Withers, 1977). Resting rates of O_2 consumption (RMR) for each bird represented the mean of the two lowest 10 min averages of O_2 uptake recorded in two separate sampling periods during the 8 h measurement period (Buttemer *et al.* 1991). RQ (CO_2 production/O_2 consumption) were based on 8 h averages of CO_2 production and O_2 consumption.

Extraction and analysis of lipids

Total lipids were extracted from 10 g of homogenised muscle tissue with chloroform–methanol (2:1, v/v) containing 0·01 % (v/v) butylated hydroxytoluene following procedures described by Folch *et al.* (1957) and Ashes *et al.* (1992). Fatty acid methyl esters were prepared from an aliquot of the extracts using the toluene–H_2SO_4 procedure described by Christie (1989). This procedure was also used to prepare fatty acid methyl esters from abdominal fat tissue (50–100 mg), diet samples (500 mg) and individual dietary fat additives (500 mg). Individual fatty acids were separated and quantified by GC (Perkin Elmer Autosystem, FID and PE Nelson data system, model 10202, Perkin Elmer Pty Ltd, CT, USA), fitted with a BPX 70 capillary column (50 mm × 0·25 mm; SGE Australia Pty Ltd. Ringwood, Victoria, Australia). He was used as the carrier gas, with an injection split ratio of 100:1. The GC was temperature programmed from 150 °C to 210 °C at a rate of 2 °C/min, with an injection temperature of 210 °C and a detector temperature of 250 °C. Peaks separated were identified by comparison with standard samples of known composition.

Water and fat content of the breast muscle

Water and fat contents were determined using tissue samples taken from eight of the ten birds selected at random from each dietary treatment. The frozen tissue samples were allowed to thaw to room temperature, weighed and dissected into 1 mm pieces before being placed in cellulose extraction thimbles (single thickness, 28 mm i.d. × 80 mm length; Whatman Int. Ltd., Maidstone, Kent, England, catalogue no. 2800288) that had been previously dried at 60°C. To determine muscle water content, muscle tissues were incubated at 60°C for 24 h, weighed and further incubated at 60°C until tissue weights had stabilised. The fat content of the dried muscle was determined gravimetrically following 24 h lipid extraction in light petroleum (b.p. 60–80°C) using a Soxhlet apparatus.

Statistical analysis

All data are presented as means with their standard errors. Statistical examination of treatment effects was determined

Table 3. Growth performance and carcass characteristics of chickens fed fish oil-, sunflower oil- or tallow-supplemented diets*

(Values are means with their standard errors for ten birds per treatment group)

Treatment group...	Fish oil		Sunflower oil		Tallow	
	Mean	SE	Mean	SE	Mean	SE
Feed intake (g/bird)	5212	213	5225	218	5531	175
Initial body wt (g/bird)	584	26	582	31	608	20
Wt gain (g/bird)	2714	121	2808	151	2712	129
Feed:gain (g/g)	1·92[ab]	0·04	1·87[a]	0·02	2·05[b]	0·04
Fat pad (g/bird)	45·3[c]	7·2	42·2[c]	8·3	107·4[d]	10·6
Breast muscle: g/bird	572	20	585	30	542	28
Water (%)	24·3[a]	1·5	24·1[a]	2·1	19·1[b]	0·9
Fat (%)	0·63	0·02	0·72	0·13	0·88	0·28
Breast muscle:fat pad	15·8[c]	3·1	16·9[c]	3·2	5·9[d]	0·9

[a,b,c,d]Mean values in the same row with unlike superscript letters were significantly different: [a,b]$P<0·05$; [c,d]$P<0·01$.
* For details of diets and procedures, see p. 12 and Tables 1 and 2.

by ANOVA and Tukey-Kramer multi-comparison test. For voluntary food intake repeated-measures ANOVA was used to analyse for the effects of treatment, time and their interaction (CLR ANOVA program; Clear Lake Research Inc., Houston, TX, USA).

Results

Growth performance and carcass characteristics

There were no significant differences between male and female birds for any of the variables measured, and consequently data for the two genders were pooled. Feed intake was statistically indistinguishable between the three treatments (Table 3), but the chickens fed the tallow diet tended to consume more feed compared with either the sunflower oil- or fish oil-fed chickens. The weight gain of the birds was not affected by dietary treatment, but feed: gain for the fish oil-fed group was lower than that for the follow-fed group, and for the sunflower oil-fed group it was significantly lower ($P<0·05$) than that for the tallow-fed group. Moreover, birds fed sunflower oil and fish oil diets had significantly smaller abdominal fat pads ($P<0·01$) when compared with the chickens fed the tallow diet. Birds fed the fish oil and sunflower oil tended to have the largest and leanest breast muscle mass; however, values did not differ significantly between the dietary treatments. The significantly lower abdominal fat pad mass and the larger breast muscle mass for the two PUFA groups resulted in a significantly greater breast muscle:abdominal fat ($P<0·01$) compared with that for the chickens fed tallow. While birds fed fish oil and sunflower oil diets had significantly higher water contents of breast muscle ($P<0·05$) than tallow-fed birds, fat content did not differ significantly between diets, although the fat content tended to be higher for the tallow-fed chickens.

Fatty acid content of the abdominal fat pad

The fatty acids found in abdominal fat pads (Table 4) reflected the dietary fatty acid profile. The fatty acids EPA and DHA were only found in the fat pads from chickens fed fish oil. The relative proportions of tissue incorporation of these fatty acids were similar to the dietary proportion of EPA and DHA; the percentage incorporation being 4·7 and 4·4 respectively. Feeding either fish oil or sunflower oil diets significantly reduced the proportion of palmitic and (16 : 0) oleic acids (18 : 1) in fat pads compared with feeding tallow ($P<0·05$). In contrast, fat pads from chickens fed the sunflower oil diet had a significantly higher ($P<0·01$) proportion of linoleic acid (18 : 2*n*-6) compared with feeding either fish oil or tallow. The P:S for the abdominal fat pad of the tallow-fed group was lower (0·51) than that of the fish oil dietary group (0·81) while, consistent with the dietary fatty acid values, the P:S was highest for the chickens fed the sunflower oil diet (2·17).

Fatty acid composition of breast muscle

The fatty acid profiles in breast muscle for the three experimental dietary treatments showed many similarities to the abdominal fat pad profiles (Table 5). However, unlike the abdominal fat pad, some of the fatty acids observed in the muscle were not present in the diet. A most notable difference was the level of arachidonic acid (20 : 4*n*-6) in muscle tissue. The proportion of arachidonic acid, in breast muscle was significantly lower ($P<0·01$) in the fish oil group than in birds fed either sunflower oil or tallow. The high concentration of linoleic acid (18 : 2*n*-6) in the sunflower oil diet was reflected in its significantly greater incorporation into muscle compared with the other dietary groups ($P<0·01$). Feeding fish oil significantly increased ($P<0·01$) the proportion of breast muscle DHA compared with the sunflower oil and tallow diets. However, consistent with the fatty acid values for, the abdominal fat pad EPA was observed only in the muscle tissue of the birds fed fish oil. DHA:EPA was higher in the muscle of the chickens fed the fish oil, and this finding was the inverse of what was evident in both the diet and the abdominal fat pad. The P:S values for breast muscle were similar to those in abdominal fat pads for the three dietary groups, being lower for the tallow-fed chickens (0·65) compared with either fish oil (0·94) or sunflower oil feeding (1·51).

Table 4. Fatty acid composition (mol/100 mol) of the abdominal fat pad of chickens fed fish oil-, sunflower oil- or tallow-supplemented diets†

(Mean values with their standard errors for eight birds per treatment group)

	Fish oil		Sunflower oil		Tallow	
Fatty acid	Mean	SE	Mean	SE	Mean	SE
14 : 0	3·74[a]	0·09	0·47[b]	0·04	0·94[c]	0·01
16 : 0	19·86[a]	0·27	14·27[b]	0·18	24·33[c]*	0·26
16 : 1c	7·53[a]	0·12	1·83[b]	0·21	4·46[c]	0·18
18 : 0	5·63	0·18	6·26	0·36	5·57	0·11
18 : 1 *cis* 9	21·75[a]	0·72	29·13[b]	0·53	43·64[c]*	0·26
18 : 1 *cis* 7	2·94[a]	0·06	0·59[b]	0·02	1·33[c]*	0·09
18 : 2*n*-6	12·38[a]	0·40	44·04[b]**	0·61	14·80[c]	0·28
18 : 3*n*-3	1·15[a]	0·02	0·58[b]	0·02	0·77[c]	0·02
20 : 0	1·87[a]	0·04	0·15[b]	0·01	0·22[c]	0·01
20 : 1	0·94	0·01	ND		ND	
20 : 5*n*-3	7·31	0·3	ND		ND	
22 : 6*n*-3	4·42	0·3	ND		ND	
Total saturated (S)	31·1		21·2		31·1	
Total monounsaturated	33·9		31·7		49·7	
Total polyunsaturated (P):*n*-6	12·9		44·0		14·8	
n-3	12·9		0·6		0·8	
P:S‡	0·81		2·17		0·51	

ND, not detected.
[a,b,c]Mean values in the same row with unlike superscript letters were significantly different ($P<0·05$).
Mean values were significantly different from those for the other treatment groups: *$P<0·05$, **$P<0·01$.
† For details of diets and procedures, see p. 12 and Tables 1 and 2.
‡ *n*-6 + *n*-3/14 : 0 + 16 : 0 + 18 : 0 + 20 : 0.

Plasma metabolites, metabolic rate and RQ

Values for selected plasma metabolites, RMR and RQ for the three dietary treatments are shown in Table 6. The plasma triacylglycerol concentrations tended to be lower in the birds fed the sunflower oil diet, and were significantly lower ($P<0·05$) in the fish oil group when compared with birds fed tallow. Plasma non-esterified fatty acid concentrations did not differ between groups. Fish oil-fed chickens had lower total plasma cholesterol concentrations than those of birds fed either sunflower oil or tallow. Plasma glucose concentrations did not differ between the three dietary

Table 5. Fatty acid composition (mol/100 mol) of the breast muscle in chickens fed fish oil-, sunflower oil- or tallow-supplemented diets†

(Mean values with their standard errors for eight birds per treatment group)

	Fish oil		Sunflower oil		Tallow	
Fatty acid	Mean	SE	Mean	SE	Mean	SE
14 : 0	2·78[a]	0·3	ND		0·82[b]	0·10
16 : 0	20·79[a]	0·20	15·91[b]	0·50	23·36[c]	0·21
16 : 1c	4·37[a]	0·14	1·05[b]	0·10	2·58[c]	0·24
18 : 0	9·92	0·50	10·41	0·50	9·25	0·53
18 : 1 *cis* 9	16·65[a]	0·39	20·47[a]	0·94	31·89[b]	2·01
18 : 1 *cis* 7	3·13[a]	0·13	1·73[b]	0·12	2·59[a]	0·17
18 : 2 *n*-6	10·1[a]	0·49	31·29[b]**	1·81	14·84[c]	0·40
18 : 3 *n*-3	0·93	0·12	ND		ND	
20 : 0	1·35	0·10	ND		ND	
20 : 4*n*-6	2·14[a]**	0·20	8·51[b]	0·81	5·99[b]	0·89
20 : 5*n*-3	7·21	0·32	ND		ND	
24 : 0	ND		1·66	0·37	1·22	0·31
24 : 1	3·22[a]	0·18	1·24[b]	0·24	0·91[b]	0·14
22 : 6*n*-3	12·29[a]**	0·90	2·30[b]	0·40	1·60[b]	0·26
Total saturated (S)	34·8		27·9		34·7	
Total monounsaturated	27·4		24·5		37·9	
Total polyunsaturated (P):*n*-6	12·2		39·8		20·8	
n-3	20·4		2·3		1·6	
P:S	0·94		1·51		0·65	

ND, not detected.
[a,b,c]Mean values in the same row with unlike superscript letters were significantly different ($P<0·05$).
Mean values were significantly different from those for the other treatment groups: **($P<0·01$).
† For details of diets and procedures, see p. 12 and Tables 1 and 2.
‡ *n*-6 + *n*-3/14 : 0 + 16 : 0 + 18 : 0 + 20 : 0 + 24 : 0.

Table 6. Plasma metabolite and insulin concentrations, RQ and resting metabolic rate (RMR) of chickens fed fish oil-, sunflower oil- or tallow- supplemented diets*

(Values are means with their standard errors for ten birds per treatment group, except RQ and RMR, where five birds were used.

	Fish oil		Sunflower oil		Tallow	
	Mean	SE	Mean	SE	Mean	SE
Triacylglycerols (mmol/l)	0·24[a]	0·05	0·28[ab]	0·03	0·42[b]	0·08
NEFA (mmol/l)	0·20	0·04	0·15	0·02	0·13	0·02
Cholesterol (mmol/l)	2·40	0·11	2·77	0·17	2·81	0·17
Glucose (mmol/l)	13·7	0·3	13·9	0·4	13·4	0·3
RQ	0·89	0·04	0·84	0·02	0·92	0·02
RMR (ml O_2/g body mass per h)	1·019	0·035	1·084	0·087	0·938	0·038

NEFA, non-esterified fatty acids.
[a,b]Mean values in the same row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and treatments, see p. 12 and Tables 1 and 2.

groups. The mass-specific RMR tended to be elevated in birds fed sunflower oil and fish oil compared with the tallow-fed group. In contrast, the RQ for chickens fed sunflower oil tended to be lower than that of birds fed tallow, and birds fed fish oil had RQ values intermediate to those of these groups.

Discussion

A major finding of the present study was that broilers fed diets containing 80 g fish oil (*n*-3 PUFA) or sunflower oil (*n*-6 PUFA)/kg had significantly ($P<0·01$) smaller abdominal fat pad mass than birds fed tallow, a more saturated fat. Fat pad mass made up 1·1 and 0·8% of the body weight for the fish oil and the sunflower oil dietary groups respectively, compared with 2·7 % for the tallow diet. A similar finding has also been reported for female broilers that were fed sunflower oil for 32 d (Sanz *et al.* 2000). Abdominal fat pad mass has been shown to be highly correlated with the total fat content of both the carcass and the edible meat of chickens (Becker *et al.* 1979; Akiba *et al.* 1995). Thus, the significant reduction in the abdominal fat pad mass for the broilers fed the fish oil and sunflower oil diets presumably reflects a lower total body fat content, and demonstrates the importance of fatty acids in modulating body fat. In addition, the lower metabolisable energy of the tallow diet relative to the more-highly-absorbed oils (Annison, 1974) resulted in a higher crude protein:metabolisable energy value for the tallow diet compared with the other diets. Increased crude protein:metabolisable energy is usually associated with lower body fat deposition (Lewis, 1978); therefore, the effect of dietary PUFA on body component partitioning may be even greater than that indicated by the data.

In the present study the decrease in the fat pad mass for the two PUFA dietary groups was associated with an increase in lipid oxidation, as indicated by the lower resting RQ observed for the chickens fed fish oil or sunflower oil compared with those fed tallow. This finding is consistent with earlier results showing preferential mobilisation and/or oxidation of more-unsaturated lipids (Halminski *et al.* 1991; Raclot & Groscolas, 1993). A change in lipid partitioning has also been reported for human subjects in whom the dietary inclusion of *n*-3 PUFA for 3 weeks reduced body fat mass and increased basal lipid oxidation (Couet *et al.* 1997). Similar results have been obtained with rodents (Hill *et al.* 1993; Su & Jones, 1993). Taken together with the present results these findings suggests that dietary fatty acid profile can modulate catabolic and anabolic functions.

The differences in metabolic rate and body composition of birds fed fish oil and sunflower oil compared with those fed tallow may be influenced by more than just dietary P:S. They may relate to the percentage of dietary monounsaturated fatty acids or to ingestion of specific fatty acids, or a combination of both. For example, the abdominal fat pad and breast muscle tissues from birds fed tallow contained a significantly higher percentage of monounsaturated fatty acids than those from birds fed fish oil or sunflower oil. These differences in tissue fat type reflect the higher percentage of monounsaturated fatty acids in the tallow diet compared with the other diets. Doucet *et al.* (1998) reported that the waist circumference of human subjects was directly correlated with total dietary saturated and monounsaturated fat content but not with PUFA content in the diet.

The reduced plasma triacylglycerol concentrations and slightly enhanced plasma non-esterified concentrations observed for the PUFA dietary groups are concordant with animals that are oxidising lipid (Newman *et al.* 1998). The lower plasma triacylglycerol concentrations observed in broilers fed fish oil agrees with the findings of other studies in chickens (Phetteplace & Watkins, 1990; Leskanich & Noble, 1997), rats (Chait *et al.* 1974; Shimomura *et al.* 1990; Okuno *et al.* 1997) and man (Toft *et al.* 1995). The present study demonstrated that reduced circulating triacylglycerol concentrations were not specific to *n*-3 PUFA feeding, since birds fed sunflower oil also had reduced plasma triacylglycerol concentrations; a similar finding has been reported previously for chickens (Sanz *et al.* 2000) and man (Weintraub *et al.* 1988).

The decrease in plasma triacylglycerol and the reduced RQ observed in the chickens fed the fish oil and sunflower oil diets may also be a response to the action of specific fatty acids to stimulate enzymes of the β-oxidative pathway. Fish oil feeding enhances carnitine palmitoyltransferase-1

activity in rats (Berge *et al.* 1988), mice (Borgeson *et al.* 1989) and Syrian hamsters (Surette *et al.* 1992), but decreases the sensitivity of carnitine palmitoyltransferase-1 to malonyl-CoA inhibition in liver mitochondria (Wong *et al.* 1984). In addition, sunflower oil feeding stimulates the activity of both carnitine palmitoyltransferase-1 and *S*-3-hydroxyacyl-CoA dehydrogenase in chickens (Sanz *et al.* 2000). Thus, an increase in carnitine palmitoyltransferase-1 activity and/or reduction in its malonyl-CoA inhibition would render fatty acids more available for β-oxidation. Support for preferential oxidation of *n*-3 and *n*-6 PUFA over saturated fatty acids has been demonstrated (Leyton *et al.* 1987). In Leyton *et al.* (1987) *n*-3 PUFA were oxidised for energy in higher proportions than *n*-6 PUFA, which in turn were used for energy more readily than the saturated fats which were preferentially stored. The preference for fatty acids to be either oxidised or stored was found to be dependent on chain length and degree of saturation.

The higher RMR of broilers fed the fish oil and sunflower oil diets may be a consequence of the greater proportion of metabolically-active tissues in these treatment groups. In addition to the greater fat-free mass, these two groups also had a larger breast muscle mass compared with the chickens fed the tallow diet. This result is consistent with that of Chappell *et al.* (1999), who showed a significant correlation ($P < 0{\cdot}003$) between BMR and breast muscle mass. Alternatively, the increased RMR could be due to an increase in ion 'leakage' following activation of Na^+/K^+-ATPase induced through an increase in the PUFA content of cell membranes (Else & Hulbert, 1987).

The fatty acid composition of both the breast muscle and the abdominal fat pad reflects the dietary fat profile, which is consistent with findings of other research (Jen *et al.* 1971; Phetteplace & Watkins, 1989; O'Neil *et al.* 1998). In the present study the feeding of fish oil increased the proportion of the long-chain *n*-3 PUFA EPA and DHA, but decreased the proportion of arachidonic acid. However, the proportion of DHA relative to that of EPA in the muscle was greater than those of the diet and fat pad, suggesting a preferential incorporation of DHA into muscle tissue. Feeding sunflower oil increased the proportion of linoleic and arachidonic acids, whereas the feeding of tallow increased the proportion of palmitic and oleic acids. The greatest concentration of arachidonic acid was observed in the breast muscle of birds fed sunflower oil, and was presumably synthesised from linoleic acid either within muscle tissue or the liver and delivered to the muscle. This result was not surprising, as the biosynthesis of arachidonic acid in mammalian cells occurs through a sequence of alternating desaturation and chain-elongation reactions using linoleic acid as the initial substrate (Watkins, 1995).

The present investigation demonstrates the effects that changes in dietary fatty acid profile have on tissue fatty acid composition. Feeding *n*-3 and *n*-6 PUFA resulted in a leaner bird, with an accompanying improvement in feed conversion efficiency, both important criteria for economically-sustainable animal production systems. These effects reflect changes in avian metabolism through the modulation of lipid deposition and oxidation by *n*-3 and *n*-6 PUFA, and concur with the results of studies in rats and man. In addition, the incorporation of these fatty acids into the tissues of birds could impact favourably on the health of consumers.

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