

## Dietary *n*-3 and *n*-6 fatty acids alter avian metabolism: molecular-species composition of breast-muscle phospholipids

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The effects of diets high in *n*-3 polyunsaturated fatty acids (PUFA; provided by fish oil), *n*-6 PUFA (sunflower oil) or in more-saturated fatty acids (tallow) on the distribution of subclasses of choline phospholipids (PC) and ethanolamine phospholipids (PE) from the breast muscle of broiler chickens were examined. Supplementation with the different fatty acids had no effect on the distribution of phospholipid subclasses. Feeding sunflower oil or tallow gave a molecular-species profile similar in both fatty acid subtype and proportion. In the diacyl PC phospholipids, 16:0–18:1*n*-9 and 16:0–18:2*n*-6 accounted for approximately 60% of the total molecular species, whereas for the alkylenyl PC the predominant species were 16:0–18:1*n*-9 and 16:0–20:4*n*-6. Of the diacyl PE the dominant species was 18:0–20:4*n*-6 which accounted for 50% of the molecular species, and of the alkylenyl PE the dominant species were 16:0–18:1*n*-9, 16:0–20:4*n*-6 and 18:0–20:4*n*-6. Supplementation with fish oil significantly increased levels of both eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3) in PC and PE when compared with either sunflower oil or tallow supplementation. The increase in the *n*-3 PUFA incorporation was associated with a corresponding decrease in the proportion of arachidonic acid (20:4*n*-6) in both PC and PE. Different dietary fats induce different patterns of fatty acid incorporation and substitution in the *sn*-2 position of the diacyl and alkylenyl PC and PE of avian breast muscle, and this finding is indicative of selective acyl remodelling in these two phospholipids.

**Chicken: Ethanolamine phospholipids: Choline phospholipids: Polyunsaturated fatty acids**

Cell membranes modulate a variety of cellular functions, including receptor-mediated signal transduction, the processing of receptor ligand complexes, the passage of hydrophobic effectors such as steroid hormones that interact with their cytoplasmic membrane receptors, and the passage and transport of ions and nutrients (Birnbaumer *et al.* 1977; Robertson, 1983; Steinman *et al.* 1983; Hulbert & Else, 2000); factors critical to normal cellular function. Although membrane composition and functional properties are regulated by genetic and environmental components, it has become increasingly clear that membrane lipids, particularly the fatty acyl chains play a key role in determining cellular function (see Hulbert & Else, 1999).

Diet is a major environmental factor, and alteration of the dietary fatty acid profile affects several physiological processes, including the lowering of plasma lipid concentrations, modulation of eicosanoid formation and cell membrane properties (Harris, 1989; Kinsella *et al.* 1990). Although *n*-3 polyunsaturated fatty acids (PUFA) have received greater attention because of their perceived health benefits, consumption of *n*-6 PUFA, specifically linoleic acid (18:2*n*-6; LA), also modulates metabolism by reducing lipid secretion (Strum-Odin *et al.* 1987) and enhancing both insulin binding and insulin action (Field *et al.* 1990; Cheema *et al.* 1992). Fatty acids such as eicosapentaenoic acid (20:5*n*-3; EPA), docosahexaenoic acid

**Abbreviations:** AA, arachidonic acid (20:4); DHA, docosahexaenoic acid (22:6); EPA, eicosapentaenoic acid (20:5); LA, linoleic acid (18:2); PC, choline phospholipids; PE, ethanolamine phospholipids; PUFA, polyunsaturated fatty acid.

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(22:6*n*-3; DHA) and arachidonic acid (20:4*n*-6; AA) are absorbed and incorporated into the subclasses (diacyl and alkylenyl) of phospholipids containing choline (PC) and ethanolamine (PE) in muscle of ruminants (Scott *et al.* 1993) and single-stomached animals (dogs; Gross, 1985)

The dietary fat profile has also been implicated in modulating the body fat content of chickens (Sanz *et al.* 2000; Newman *et al.* 2002), rodents (Cunnane *et al.* 1986; Shimomura *et al.* 1990; Cheema *et al.* 1992) and man (Couet *et al.* 1997). The feeding of specific fat types influences the composition of body-weight gain. For example, feeding *n*-3 PUFA reduces body-weight gain in genetically-obese *ob/ob* mice (Cunnane *et al.* 1986), while dietary *n*-6 PUFA reduce body fat in rats (Shimomura *et al.* 1990), and diets high in saturated fatty acids lead to an increase in body fatness when compared with highly-enriched PUFA diets (Shimomura *et al.* 1990; Pan *et al.* 1994). These responses correspond to changes in tissue phospholipid-fatty acid composition that reflect the dietary fatty acid profile (Pan & Storlien, 1993). Changes in phospholipid composition may alter the physical and chemical dynamics of the cell membrane (Clandinin *et al.* 1991), and this change may in turn influence processes involved in energy metabolism.

Newman *et al.* (2002) demonstrated that dietary fat source (fish oil, sunflower oil or tallow) alters abdominal fat deposition and intermediary metabolism in broiler chickens. A reduction in body fat content occurred when the dietary fat was predominately PUFA. In view of the relationship between dietary fatty acids, tissue phospholipids and body composition (Pan & Storlien, 1993), we characterised the major molecular species of PC and PE subclasses (diacyl and alkylenyl; Scott *et al.* 1993; Schmid *et al.* 1995; Hermier *et al.* 1999) in breast muscle samples obtained from birds fed fish oil, sunflower oil and tallow according to Newman *et al.* (2002), to determine the form of remodelling that occurs when feeding different sources of PUFA.

## Materials and methods

### Birds, diets and study design

Details of the experimental design, diets and birds, including management and collection of samples have been described by Newman *et al.* (2002). The diets contained 80 g edible grade tallow (<1% (w/w) free fatty acids), sunflower oil or fish oil/kg and were fed to the chickens from age 3 to 8 weeks. The fatty acid composition of the three experimental diets is shown in Table 1. The breast muscle tissue for molecular-species analysis was obtained from four broiler chickens fed each of the experimental diets.

### Material

Phospholipase C (*Bacillus cerus* type XIII), benzoic anhydride, and 4-dimethylaminopyridine were purchased from Sigma Chemical Co. (St Louis, MO, USA). Phospholipids and diacylglycerol standards were obtained from Serdary (London, Ont., Canada). TLC plates, silica gel 60F 254

**Table 1.** 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 <i>cis</i> 9	14.07	23.27	38.03
18:2 <i>n</i> -6	19.77	62.73	25.80
18:3 <i>n</i> -3	1.81	1.13	1.49
20:0	2.94	ND	ND
20:5 <i>n</i> -3	13.41	ND	ND
22:6 <i>n</i> -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): <i>n</i> -6	19.77	62.73	25.80
<i>n</i> -3	23.59	1.13	1.47
P:S†	1.57	4.97	0.84

ND, not detected.

\* For details of diets, see Newman *et al.* (2002).

†  $n-6 + n-3/14:0 + 16:0 + 18:0 + 20:0$ .

(200 mm × 200 mm), were obtained from E. Merck (Darmstadt, Germany). The solvents used for the TLC solvent system were AR grade and those for HPLC separation were HPLC grade, and they were supplied by Mallinckrodt, Selby Biolab (Sydney, NSW, Australia).

### Lipid extraction

Total lipids were extracted from 10 g muscle tissue with chloroform–methanol (2:1, v/v) containing 0.01% (v/v) butylated hydroxytoluene. The tissue was macerated for 2 min using a Waring blender, equilibrated for 4 h at room temperature then filtered (Watman no. 1 filter paper). The filtrate was washed with water (Folch *et al.* 1957) and the phases allowed to separate before aspirating and discarding the top phase. The bottom layer was retained, evaporated to dryness and redissolved in chloroform–methanol (2:1, v/v).

### Separation of phospholipids and neutral lipids

Glass columns (20 ml) were prepared by loading with 3–5 g silicic acid suspended in 10–15 ml chloroform. The lipid samples were dissolved in chloroform (0.5 ml), applied to the top of the column and then eluted with 60 ml chloroform to separate the neutral lipids. The phospholipids were then eluted with 60 ml methanol and their concentration was determined from P estimation using a factor of  $P \times 25$  (Bartlett, 1959).

### Separation of total phospholipids into individual phospholipid classes

All remaining phospholipid extracts were evaporated to dryness and then redissolved in 100–200  $\mu$ l chloroform–methanol (2:1, v/v). Samples and phospholipid standards were separated by TLC using silica gel G plates that had been activated by incubating at 100°C for 1 h. The plates were then run in solvent system no. 1 (60 ml chloroform – 25 ml methanol – 50 ml acetone – 2 ml acetic acid –

5 ml water), air-dried for 20–30 min and then dried under vacuum for 30 min. The silica plates were then run in the same direction in solvent system no. 2 (60 ml chloroform – 50 ml methanol – 10 ml acetic acid – 3.3 ml water). Individual phospholipids and standards were initially visualised by spraying the plates with ninhydrin, incubating at 100°C and then re-spraying to detect P (Vaskovsky *et al.* 1975). Final visualisation of the lipids was achieved by spraying the plate with 2,7-dichlorofluorescein (0.02% (w/v) in ethanol) and then exposing it to u.v. light. Areas of gel corresponding to the phospholipids were scraped off the plate and extracted with chloroform–methanol–water (5:5:1, by vol.). The phospholipid extracts were washed three times in water and the volumes were adjusted to 10 ml with chloroform–methanol (2:1, v/v). A portion of the extract was used to prepare fatty acid methyl esters (Christie, 1989) and these derivatives were separated and quantified using GLC (Perkin Elmer Autosystem, FID and PE Nelson data processing 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 GLC 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. A portion of the phospholipid extract was used to determine the amount of phospholipid-P and the remainder was used for analysis of molecular species.

#### *Separation of molecular species*

The purified phospholipids were evaporated to dryness, redissolved in 10 mM-Tris hydrochloride (pH 7.5; 1.25 ml) containing 30 mM-H<sub>3</sub>BO<sub>3</sub> and 10 mM-CaCl<sub>2</sub> then sonicated in a cold water bath (2°C) for 5 min. Individual phospholipids (1–2 mg) were hydrolysed with 50 units phospholipase C, then extracted with 2 ml diethyl ether, mixed by vortexing and incubated overnight at room temperature, while being constantly agitated. The diethyl ether layer was removed and the released 1,2-diacylglycerols were extracted in diethyl ether and dried under N<sub>2</sub> (Blank *et al.* 1984).

#### *Benzoate derivatives*

The extracted and dried diacylglycerols were dissolved in 0.3 ml benzene containing benzoic anhydride (10 mg) and 4-dimethylaminopyridine (4 mg) and allowed to react for 2 h. The benzoate derivatives were extracted three times with 2 ml hexane and separated into three subclasses (diacyl, alkylacyl and alkylenyl) by TLC on silica gel G in a solvent system of toluene–hexane–diethyl ether (50:45:5, by vol.). The diacylglycerolbenzoates were visualised under u.v. light and the areas of gel corresponding to these derivatives were scraped from the plates and extracted with hexane. The diacylglycerol extract was evaporated to dryness, and the diacylglycerol redissolved in acetonitrile–2-propanol (80:20, v/v), and the distribution

of the three subclasses in the PE and PC was determined by measuring the absorbance at 230 nm.

#### *Separation by HPLC*

The molecular species of the diacyl and alkylenyl derivatives were separated using a Waters (Milford, CT, USA) HPLC system, comprising a 501 pump, a 484 u.v. detector (operating at 230 nm), and a 740 data module. An SGE ODS 2–8/5 column (5 µm, 250 mm × 4 mm i.d.; SGE Australia Pty Ltd) was used at an operating temperature of 24°C. Samples of the benzoate derivatives were positioned using two solvent systems, acetonitrile–2-propanol (80:20, v/v) and methanol–2-propanol (95:5, v/v). The molecular species were separated by isocratic elution at a flow rate of 1.0 and 0.8 ml/min respectively. A 12:0–12:0 diacylglycerolbenzoate derivative was used as an internal standard.

#### *Identification of molecular species*

Identification of the individual molecular species was achieved by a combination of HPLC analysis of standards, plotting their relative retention times using the methods outlined by Takamura *et al.* (1986), Bell (1989) and Takamura & Kito (1991), and GLC analysis of the fatty acid methyl esters of the peaks corresponding to the eluted species.

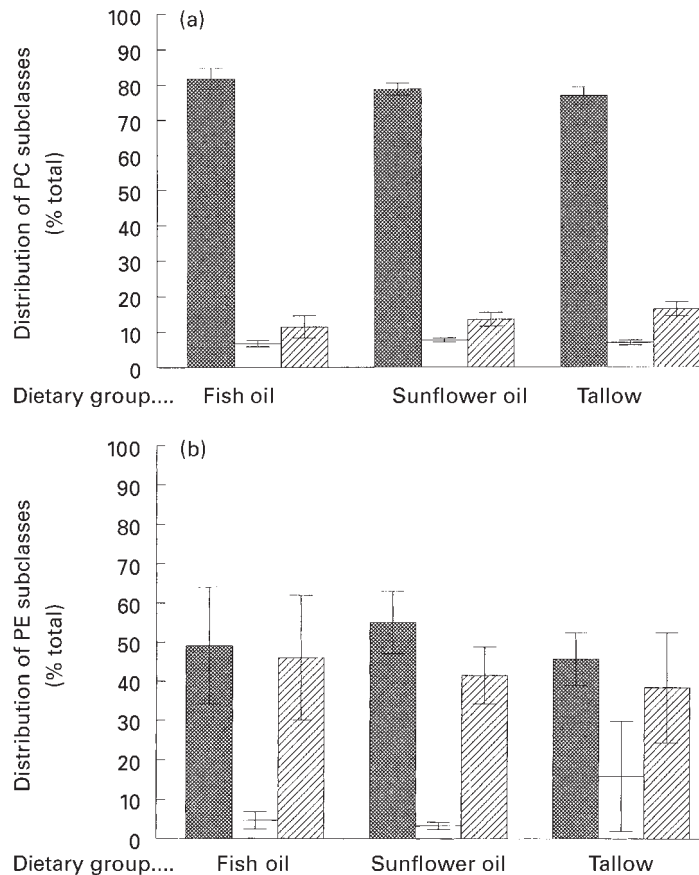
## **Results**

#### *Distribution of choline phospholipid subclasses in muscle*

The percentage distribution of the PC subclasses (diacyl, alkylacyl and alkylenyl) for the three dietary treatments is shown in Fig. 1(a). The diacyl subclass accounted for the highest percentage of the PC fraction. The feeding of the different dietary fats had no significant effect on the distribution of the PC subclasses. For birds fed the fish oil diet the diacyl subclass comprised 81.8 (SE 2.9) % and for the sunflower oil- and tallow-fed chickens the corresponding values were 78.9 (SE 1.8) and 76.8 (SE 2.5) % respectively. The percentage distributions of the alkylacyl and alkylenyl subclass for the three dietary groups were respectively, 6.7 (SE 0.9) and 11.5 (SE 3.1) for the fish oil group, 7.71 (SE 0.6) and 13.5 (SE 1.9) for the sunflower oil group and 6.9 (SE 0.7) and 16.3 (SE 1.9) for the tallow group.

#### *Distribution of ethanolamine phospholipid subclasses in muscle*

The percentage distribution of the PE subclasses (diacyl, alkylacyl and alkylenyl) for the three dietary treatments is shown in Fig. 1(b). As for the PC subclasses, the feeding of the dietary fats had no significant effect on the distribution of the PE subclasses. However, unlike those for the PC subclasses the percentage distributions for the PE diacyl and the alkylenyl subclasses were similar. Feeding fish oil to chickens resulted in a percentage distribution of 49.2 (SE 14.9) and 46.1 (SE 15.9) for the diacyl and



**Fig. 1.** Distribution of (a) choline phospholipid (PC) subclasses and (b) ethanolamine phospholipid (PE) subclasses (diacyl (▨), alkylacyl (□) and alkylenyl (▩)) in breast muscle from chickens fed fish oil, sunflower oil or tallow. For details of diets and procedures, see p. 20. Values are means with their standard errors represented by vertical bars.

alkylenyl subclasses respectively. Similarly, the percentage distributions for the diacyl and alkylenyl subclasses were respectively 55.2 (SE 8.0) and 41.5 (SE 7.3) for the sunflower oil group and 45.7 (SE 6.8) and 38.4 (SE 14.1) for the tallow group. The alkylacyl subclass was present at a significantly lower ( $P < 0.01$ ) level with all dietary treatments when compared with both the diacyl and alkylenyl subclasses.

#### Molecular species composition

**Choline phospholipid diacyl subclass.** The composition of the molecular species of the PC diacyl subclass for breast muscle is shown in Table 2. The predominant molecular species observed were 16:0–18:1*n*-9 and 16:0–18:2*n*-6. For the fish oil, sunflower oil and tallow groups these two species accounted for (%) 37.2 (SE 3.9) and 13.7 (SE 1.2), 30.45 (SE 6.7) and 27.3 (SE 2.2) and 40.6 (SE 5.1) and 28.8 (SE 5.1) % respectively. The percentage of 16:0–18:1 was similar for all three dietary treatments. However, 16:0–18:2 was significantly reduced ( $P < 0.01$ ) in the muscle from birds fed fish oil when compared with that from birds fed either sunflower oil or tallow. Fish oil feeding resulted in a sixteenfold increase in the percentage of 16:0–20:5*n*-3 (7.9 (SE 2.7)) and a fivefold increase in

that of 16:0–22:6*n*-3 (11.1 (SE 2.0)) when compared with chickens fed the sunflower oil diet, and this increase was slightly greater than that for chickens fed tallow. The increased concentrations of 20:5*n*-3 and 22:6*n*-3 in the fish oil-fed chickens were associated with a corresponding decrease in the concentration of 20:4*n*-6 (Fig. 2(a)). Although the percentages and concentrations of the molecular species for sunflower oil- and tallow-fed chickens were similar, the molecular species 16:0–20:4 and 18:0–20:4 were significantly ( $P < 0.05$ ) elevated in the sunflower oil-fed chickens when compared with tallow-fed chickens. Feeding sunflower oil resulted in an increase in the molecular species containing two or more double bonds and a proportional decrease in molecular species containing one double bond when compared with tallow-fed chickens.

**Choline phospholipid alkylenyl subclass.** The composition of the molecular species for the PC alkylenyl subclass for the three dietary treatments is given in Table 3. The predominant molecular species in the muscle of birds fed the sunflower oil and tallow diets were 16:0–18:1*n*-9 and 16:0–20:4*n*-6. However, for chickens fed fish oil the predominant species was 16:0–22:6*n*-3. In addition, there was a significant decrease ( $P < 0.01$ ) in the concentration of 16:0–18:1 in the PC of fish oil-fed

**Table 2.** Comparison of the molecular species of the choline phospholipid diacyl subclass (mol/100 mol) in breast muscle from chickens fed fish oil-, sunflower oil- or tallow-supplemented diets\*

(Values are means with their standard errors for duplicate analysis of samples from four birds)

Dietary group...	Fish oil		Sunflower oil		Tallow	
	Mean	SE	Mean	SE	Mean	SE
Molecular species						
18:1-20:5	0.47 <sup>a</sup>	0.18	0.09 <sup>b</sup>	0.05	0.06 <sup>b</sup>	0.02
16:0-20:5	7.91 <sup>a</sup>	2.66	0.48 <sup>b</sup>	0.60	0.27 <sup>b</sup>	0.09
16:0-22:6	11.05 <sup>a</sup>	2.01	1.98 <sup>b</sup>	0.75	0.98 <sup>c</sup>	0.19
18:1-22:5	0.58 <sup>a</sup>	0.02	0.22 <sup>b</sup>	0.04	0.38 <sup>ab</sup>	0.17
18:1-20:4	ND		0.33	0.15	0.34	0.06
16:0-22:5	3.40 <sup>a</sup>	0.39	1.92 <sup>b</sup>	0.86	1.16 <sup>b</sup>	0.30
16:0-20:4	3.11 <sup>a</sup>	0.37	8.43 <sup>b</sup>	2.29	5.34 <sup>c</sup>	1.17
18:0-20:5	2.39 <sup>a</sup>	0.34	0.75 <sup>b</sup>	0.31	0.35 <sup>c</sup>	0.09
18:1-18:2	0.62	0.43	1.56	0.25	1.72	0.47
18:0-22:6	ND		ND		2.23	0.26
16:0-18:2	13.73 <sup>a</sup>	1.22	27.28 <sup>b</sup>	2.24	28.81 <sup>b</sup>	5.08
18:0-22:5	1.45 <sup>a</sup>	0.37	0.72 <sup>b</sup>	0.34	0.39 <sup>b</sup>	0.02
18:0-20:4	1.25 <sup>a</sup>	0.55	3.92 <sup>b</sup>	0.72	2.65 <sup>c</sup>	0.39
18:0-16:1	0.55	0.08	ND		ND	
18:1-18:1	1.20	0.48	1.50	0.44	2.13	0.49
18:0-18:2	3.89 <sup>a</sup>	1.11	9.53 <sup>b</sup>	2.94	6.94 <sup>b</sup>	1.11
16:0-18:1	37.18	3.90	30.45	6.66	40.45	5.14
18:0-22:4	2.83	0.75	3.07	1.23	2.33	0.84
18:0-18:1	3.35	0.75	3.39	0.63	3.27	0.39
18:0-16:0	0.43	0.13	0.94	0.76	0.37	0.14
Total	95.39		96.55		100.06	

ND, not detected.

<sup>a,b,c</sup> Mean values in the same row with unlike superscript letters were significantly different (ANOVA;  $P < 0.05$ ).

\* For details of diets and procedures, see p. 20.

chickens when compared with that of sunflower oil- and tallow-fed groups (18.6 SE 3.6, 26.9 SE 5.1 and 34.6 SE 7.8 % respectively). Conversely, the percentage of 16:0-22:6 for the fish oil group was increased sixfold when compared with that for the sunflower oil group and ninefold when compared with that for the tallow group (39.1 (SE 4.3) 6.4 (SE 0.1) and 4.1 (SE 1.1) % respectively). As was detected in the PC diacyl subclass, fish oil feeding also increased the percentage of 16:0-20:5n-3; a tenfold increase when compared with that for the sunflower oil group and a ninefold increase when compared with that for the tallow group. The increased concentrations of 20:5n-3 and 22:6n-3 were associated with a decrease in 20:4n-6 (Fig. 2(b)), as was shown for the diacyl subclass. The feeding of sunflower oil, a diet high in n-6 PUFA, resulted in a molecular-species profile containing individual fatty acids at similar percentages to those in the profile of chickens fed tallow. However, as observed in the diacyl subclass, the sunflower oil diet increased fatty acids that contained two or more double bonds and decreased species containing one double bond when compared with tallow-fed chickens.

**Ethanolamine phospholipid diacyl subclass.** The molecular species profile for the PE diacyl subclass is shown in Table 4. As observed for the PC subclasses, the molecular-species profile and their percentages were similar for both the tallow- and sunflower oil-fed chickens. However, the predominant species of the PE diacyl subclass was 18:0-20:4 for the sunflower oil- and tallow-fed chickens, and accounted for 51.1 and 46.3 % respectively. With fish

oil feeding the predominant molecular species were 18:0-20:5 and 18:0-22:6, accounting for 21.3 and 29.1 % respectively. These percentages were significantly higher ( $P < 0.01$ ) than those found in muscle from birds offered the sunflower oil or the tallow diets. As for the PC, fish oil feeding increased the concentration of the long-chain PUFA, 20:5n-3 and 22:6n-3, and significantly reduced ( $P < 0.01$ ) the concentration of 20:4n-6 when compared with the sunflower oil- and tallow-fed chickens (Fig. 2(c)).

**Ethanolamine phospholipid alkylenyl subclass.** The distribution of molecular species in the alkylenyl PE subclass for the three dietary groups is shown in Table 5. The feeding of sunflower oil and tallow resulted in profiles that were similar in species type and concentration. The dominant molecular species observed for these dietary groups were 16:0-18:1, 16:0-20:4 and 18:0-20:4, accounting for 17.9, 10.7 and 14.1 % respectively for the sunflower oil-fed chickens and 24.1, 12.7 and 10.9 % respectively for the tallow-fed group. The dominant fatty acid species for the fish oil-fed chickens were 16:0-22:6 and 18:0-22:6, and these molecular species accounted for 40.1 and 18.9 % of the alkylenyl PE subclass respectively. Consistent with the observations for the other phospholipid subclasses, the feeding of fish oil significantly increased ( $P < 0.01$ ) the proportion of the n-3 species 16:0-22:6 and 18:0-22:6 and significantly decreased ( $P < 0.01$ ) the percentage of 16:0-20:4 and 18:0-20:4 (Fig. 2(d)) compared with both sunflower oil- and tallow-fed chickens.

**Table 3.** Comparison of the molecular species of the choline phospholipid alkylenyl subclass (mol/100 mol) in breast muscle from chickens fed fish oil-, sunflower oil- or tallow-supplemented diets\*

(Values are means with their standard errors for duplicate analyses of samples from four birds)

Dietary group...	Fish oil		Sunflower oil		Tallow	
	Mean	SE	Mean	SE	Mean	SE
16:1–20:4	0.24	0.10	0.40	0.29	0.12	0.05
18:1–20:5	1.28 <sup>a</sup>	0.45	0.64 <sup>b</sup>	0.14	0.44 <sup>c</sup>	0.07
16:0–20:5	9.50 <sup>a</sup>	4.86	0.92 <sup>b</sup>	0.71	1.02 <sup>b</sup>	0.41
18:1–22:6	3.80 <sup>a</sup>	0.19	1.05 <sup>b</sup>	0.65	0.36 <sup>b</sup>	0.14
16:0–22:6	39.13 <sup>a</sup>	4.31	6.39 <sup>b</sup>	2.51	4.10 <sup>b</sup>	1.10
18:1–22:5	0.93 <sup>a</sup>	0.07	0.61 <sup>b</sup>	0.05	0.42 <sup>b</sup>	0.18
18:1–20:4	ND		8.05	1.92	6.46	1.85
16:0–22:5	4.99	0.36	ND		ND	
16:0–20:4	6.47 <sup>a</sup>	1.48	24.39 <sup>b</sup>	3.25	22.69 <sup>b</sup>	3.61
18:0–20:5	1.25 <sup>a</sup>	0.40	2.21 <sup>b</sup>	0.51	1.94 <sup>ab</sup>	0.41
18:1–18:2	ND		3.48 <sup>a</sup>	0.83	1.89 <sup>b</sup>	0.33
18:0–22:6	4.22 <sup>a</sup>	1.08	1.05 <sup>b</sup>	0.12	0.78 <sup>c</sup>	0.15
16:0–18:2	1.87 <sup>a</sup>	0.12	4.12 <sup>b</sup>	0.56	6.77 <sup>c</sup>	1.99
16:0–22:4	ND		5.06	0.90	5.85	1.00
18:0–22:5	0.33	0.05	0.76	0.39	ND	
18:0–20:4	1.83 <sup>a</sup>	0.60	5.86 <sup>b</sup>	0.87	4.04 <sup>c</sup>	0.41
18:1–18:1	1.44 <sup>a</sup>	0.37	3.28 <sup>b</sup>	0.70	3.15 <sup>b</sup>	0.55
16:0–18:1	18.55 <sup>a</sup>	3.57	26.95 <sup>b</sup>	5.07	34.58 <sup>b</sup>	7.76
18:0–18:2	1.10	0.40	1.75	0.41	1.59	0.40
18:0–22:4	0.71 <sup>a</sup>	0.33	0.93 <sup>ab</sup>	0.47	1.35 <sup>b</sup>	0.29
18:0–18:1	0.62 <sup>a</sup>	0.37	1.21 <sup>b</sup>	0.26	0.79 <sup>ab</sup>	0.23
18:0–16:0	0.09	0.05	0.11	0.07		
Total	98.33		99.22		98.31	

ND, not detected.

<sup>a,b,c</sup>Mean values in the same row with unlike superscript letters were significantly different (ANOVA;  $P < 0.05$ ).

\* For details of diets and procedures, see p. 20.

**Table 4.** Comparison of the molecular species of the ethanolamine phospholipid diacyl subclass (mol/100 mol) in breast muscle from chickens fed fish oil-, sunflower oil- or tallow-supplemented diets\*

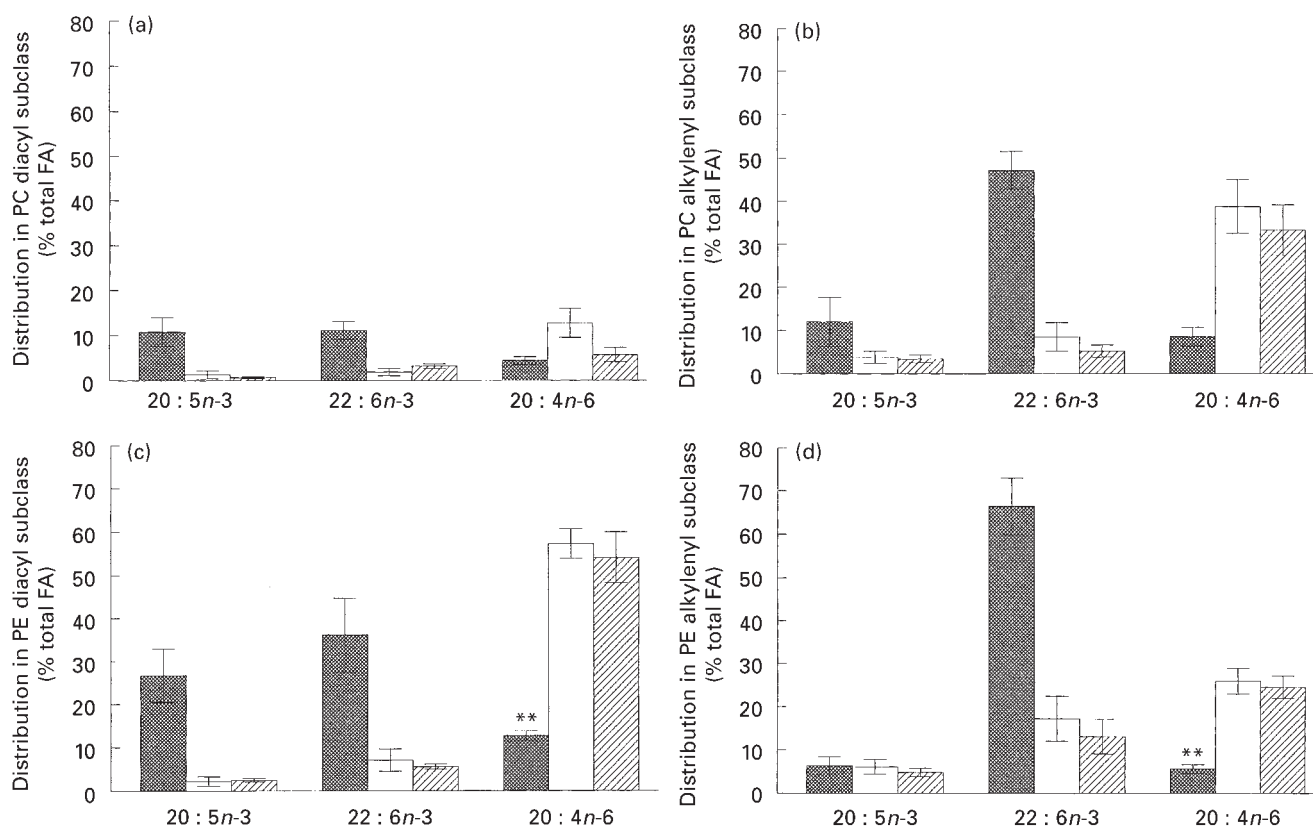
(Values are means with their standard errors for duplicate analyses of samples from four birds)

Dietary group...	Fish oil		Sunflower oil		Tallow	
	Mean	SE	Mean	SE	Mean	SE
18:1–20:5	1.45 <sup>a</sup>	0.51	ND		0.25 <sup>b</sup>	0.12
16:0–20:5	3.91 <sup>a</sup>	0.47	0.50 <sup>b</sup>	0.22	0.45 <sup>b</sup>	0.11
16:0–22:6	7.01 <sup>a</sup>	1.05	1.66 <sup>b</sup>	0.15	1.53 <sup>b</sup>	0.32
18:1–20:4	0.73	0.32	0.51	0.14	0.55	0.19
16:0–22:5	1.23 <sup>a</sup>	0.16	1.36 <sup>ab</sup>	0.49	2.09 <sup>b</sup>	0.67
16:0–20:4	1.71 <sup>a</sup>	0.40	5.65 <sup>b</sup>	0.93	7.30 <sup>c</sup>	0.46
18:0–20:5	21.31 <sup>a</sup>	5.19	1.73 <sup>b</sup>	0.87	1.79 <sup>b</sup>	0.12
18:1–18:2	1.90	0.47	ND		ND	
18:0–22:6	29.09 <sup>a</sup>	7.59	5.48 <sup>b</sup>	1.33	4.05 <sup>b</sup>	0.25
16:0–18:2	1.70 <sup>a</sup>	0.32	4.37 <sup>b</sup>	0.83	5.92 <sup>b</sup>	1.46
18:0–22:5	3.04 <sup>a</sup>	0.77	1.11 <sup>b</sup>	0.30	0.67 <sup>c</sup>	0.03
18:0–20:4	10.25 <sup>a</sup>	0.42	51.09 <sup>b</sup>	2.34	46.25 <sup>b</sup>	5.21
18:0–16:1	0.62 <sup>a</sup>	0.36	3.20 <sup>b</sup>	0.73	1.93 <sup>c</sup>	0.37
18:1–18:1	0.74	0.07	0.65	0.49	1.02	0.27
18:0–18:2	5.16 <sup>a</sup>	0.90	5.01 <sup>a</sup>	0.86	8.23 <sup>b</sup>	0.55
16:0–18:1	4.80 <sup>a</sup>	0.59	12.89 <sup>b</sup>	1.62	12.20 <sup>b</sup>	3.47
18:0–22:4	0.20	0.11	0.18	0.04	ND	
18:0–18:1	4.09 <sup>a</sup>	0.67	2.39 <sup>b</sup>	0.28	3.86 <sup>a</sup>	0.61
18:0–16:0	0.13	0.08	0.20	0.08	0.21	0.06
Total	99.81		97.98		98.30	

ND, not detected.

<sup>a,b,c</sup>Mean values in the same row with unlike superscript letters were significantly different (ANOVA;  $P < 0.05$ ).

\* For details of diets and procedures, see p. 20.



**Fig. 2.** Distribution of eicosapentaenoic acid of (20:5 $n$ -3), docosahexaenoic acid (22:6 $n$ -3) and arachidonic acid (20:4 $n$ -6) in the choline phospholipid (PC) diacyl (a) and alkylenyl (b) subclasses and in the ethanolamine phospholipid (PE) diacyl (c) and alkylenyl (d) subclasses in breast muscle from chickens fed fish oil (▨), sunflower oil (□) or tallow (▩). FA, fatty acids. For details of diets and procedures see p. 20. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the other two diets: \*\* $P < 0.01$ .

## Discussion

The present study demonstrates that the fatty acid molecular-species profile of avian breast-muscle phospholipids reflects the composition of dietary fat. Feeding fish oil to chickens increased the total content of long-chain PUFA, EPA (20:5 $n$ -3) and DHA (22:6 $n$ -3) from the  $n$ -3 series, in all subclasses of PC and PE. Feeding of sunflower oil, high in LA (18:2 $n$ -6), increased the content of the  $n$ -6 PUFA LA and AA (20:4 $n$ -6) in the phospholipid subclasses. Interestingly, the profiles of PC and PE and their subclasses in birds fed tallow were similar to those of birds fed sunflower oil, even though the concentration of LA in the tallow diet was 60% less than that in the sunflower oil diet. The samples used for this study were taken from birds described in the previous paper (Newman *et al.* 2002). In that study it was demonstrated that birds fed fish and sunflower oil had a significantly reduced abdominal fat pad mass ( $P < 0.01$ ) and lower plasma triacylglycerol levels compared with birds fed tallow. However, examination of the present data clearly shows that the remodelling of the molecular species that occurred in response to the dietary fat profile was similar for broilers fed sunflower oil and tallow, but different for those fed fish oil. The present results suggest that the changes in fat deposition and energy metabolism noted for the three dietary groups (Newman *et al.* 2002) are

not related to membrane PE and PC composition. This finding does not discount the possibility that other membrane components may be involved in the physiological changes observed.

Hazel & Williams (1990) and Schmid *et al.* (1995) suggested that the membrane bilayer is regulated to maintain a relatively constant physical state, referred to as 'homeoviscous' adaptation. This process could simply provide the cell membrane with a uniform fatty acyl environment. Individual phospholipids have the potential to 'gel out' at different temperatures, which can cause phase separation, resulting in a membrane environment consisting of solid gel patches, while other phospholipids remain in the liquid crystalline phase (Schroeder *et al.* 1998). Our results support the classical physiological structure, with the  $sn$ -1 positions occupied by a saturated fatty acid and the  $sn$ -2 positions occupied by an unsaturated fatty acid. This form of 'compulsory mixing' has been suggested by Hulbert & Else (1999) as a means of limiting phase separation in natural membranes.

In other avian species the major tissue phospholipids are PC and PE. In both Landes and Poland geese PC accounts for approximately 70% of the total phospholipid and PE accounts for approximately 25% of the total phospholipid (Hermier *et al.* 1999). The predominance of PE and PC as the major phospholipids also occurs in mammals (Schmid *et al.* 1995). Membrane studies using cultured murine

**Table 5.** Comparison of the molecular species of the ethanolamine phospholipid alkylenyl subclass (mol/100 mol) in breast muscle from chickens fed fish oil-, sunflower oil- or tallow-supplemented diets\*

(Values are means with their standard errors for duplicate analyses of samples from four birds)

Dietary group...	Fish oil		Sunflower oil		Tallow	
	Mean	SE	Mean	SE	Mean	SE
Molecular species						
16:1-20:4	0.12 <sup>a</sup>	0.03	0.10 <sup>a</sup>	0.02	0.06 <sup>b</sup>	0.01
18:1-20:5	0.78 <sup>a</sup>	0.18	0.95 <sup>a</sup>	0.20	0.44 <sup>b</sup>	0.08
16:0-20:5	3.04 <sup>a</sup>	1.21	2.30 <sup>a</sup>	0.80	0.92 <sup>b</sup>	0.24
18:1-22:6	6.44 <sup>a</sup>	2.46	1.43 <sup>b</sup>	0.67	1.79 <sup>b</sup>	0.46
16:0-22:6	40.99 <sup>a</sup>	1.89	9.35 <sup>b</sup>	1.92	7.88 <sup>b</sup>	0.99
18:1-20:4	1.47 <sup>a</sup>	0.25	1.03 <sup>b</sup>	0.02	0.83 <sup>c</sup>	0.14
16:0-22:5	3.63 <sup>a</sup>	0.22	7.87 <sup>b</sup>	0.57	8.39 <sup>b</sup>	1.04
16:0-20:4	2.30 <sup>a</sup>	0.34	10.71 <sup>b</sup>	0.99	12.73 <sup>c</sup>	0.74
18:0-20:5	2.80	0.66	2.87	0.69	3.40	0.72
18:1-18:2	ND		3.53	0.54	3.14	0.99
18:0-22:6	18.94 <sup>a</sup>	2.16	6.40 <sup>b</sup>	2.65	3.44 <sup>b</sup>	2.58
16:0-18:2	0.68 <sup>a</sup>	0.22	1.78 <sup>b</sup>	0.51	1.24 <sup>b</sup>	0.32
16:0-22:4	ND		6.22	1.20	7.22	1.20
18:0-22:5	1.51 <sup>a</sup>	0.29	2.26 <sup>b</sup>	0.57	1.94 <sup>b</sup>	0.31
18:0-20:4	1.68 <sup>a</sup>	0.38	14.08 <sup>b</sup>	2.01	10.92 <sup>c</sup>	1.66
18:1-18:1	1.10 <sup>a</sup>	0.22	3.12 <sup>b</sup>	0.89	3.88 <sup>b</sup>	0.16
16:0-18:1	10.22 <sup>a</sup>	3.19	17.88 <sup>b</sup>	3.06	24.11 <sup>c</sup>	3.77
18:0-18:2	0.78 <sup>a</sup>	0.21	2.63 <sup>b</sup>	0.50	2.71 <sup>b</sup>	0.59
18:0-22:4	0.37 <sup>a</sup>	0.08	0.10 <sup>b</sup>	0.05	0.90 <sup>b</sup>	0.04
18:0-18:1	1.11 <sup>a</sup>	0.26	2.67 <sup>b</sup>	0.25	3.13 <sup>b</sup>	0.40
18:0-16:0	0.12	0.11	ND		ND	
Total	98.08		97.25		99.05	

ND, not detected.

<sup>a,b,c</sup>Mean values in the same row with unlike superscript letters were significantly different (ANOVA;  $P < 0.05$ ).

\*For details of diets and procedures, see p. 20.

neuroblastoma cells have shown that fatty acids are rapidly incorporated and esterified into phospholipids, and that the rate of incorporation into PC is more rapid when compared with that of other phospholipids (Chakravarthy *et al.* 1986).

In the present study, LA, EPA and DHA were the principal fatty acids that paired predominantly with a 16:0 in PC, whereas AA, EPA and DHA were the principal fatty acids that were paired predominantly with 18:0 in PE. Studies in rats have shown that EPA and DHA have a high affinity for acylation to the *sn*-2 position of plasmalogen phospholipids (Blank *et al.* 1989, 1994). In addition, AA has also been shown to occur in a high proportion at the *sn*-2 position of the molecular species of diacyl PE from ruminant (Scott *et al.* 1993) and chicken (Blank *et al.* 1992) muscle. The concentration of fatty acids of the *n*-3 and *n*-6 series incorporated into the phospholipids was reflective of the dietary fatty acid concentration. Chickens fed fish oil displayed a greater proportion of EPA and DHA than AA in both PC and PE of the breast muscle. For chickens fed sunflower oil and tallow diets there was a greater proportion of LA and AA. This finding is consistent with those of other studies in chickens which show increased levels of EPA and DHA in the spermatozoan phospholipids after 30 weeks of fish oil supplementation (Kelso *et al.* 1997) and a corresponding decrease in *n*-6 fatty acids, specifically LA and AA. Similar results have also been reported in mammals; when rats were fed fish oil there was an increase in the concentrations of both EPA and DHA in the ethanolamine plasmalogens, while

the movement of AA between the lipid classes was impeded (Blank *et al.* 1994).

The *n*-3 fatty acids EPA and DHA seem to preferentially displace LA rather than oleic acid (18:1*n*-9) from the *sn*-2 position in the diacyl subclass of PC. In the alkylenyl subclass of PC, oleic acid and LA were displaced by EPA and DHA. Scott *et al.* (1993) have reported a similar substitution for oleic acid in ruminants fed fish oil; however, this substitution occurred only in the PC diacyl subclass. From the present study there is evidence of specificity of *n*-3 and *n*-6 fatty acids for different PE and PC subclasses. Concentrations of LA and AA were greater in the diacyl subclasses for both PE and PC, whereas the concentrations of EPA and DHA were found to be greater in the alkylenyl subclasses of both phospholipids.

The current study also suggests that both EPA and DHA are remodelled between the PE and PC. Remodelling of phospholipid molecular species has been shown to involve the deacylation–reacylation pathways from the combined actions of phospholipases and acyltransferases, and transacylases (Blasinde *et al.* 1995). Both EPA and DHA are present as a high proportion in the PE and PC. The high concentration of these two species is likely to be due to the dietary intake, as vertebrates are only capable of synthesising *de novo* saturated and monounsaturated chains (Hulbert & Else, 2000). Schmid *et al.* (1995) report that only a limited number of molecular species of PC and PE are remodelled by deacylation–reacylation at either the *sn*-1 or *sn*-2 position of glycerol; included



among this number is DHA. Other studies indicate that PC-DHA can be formed from the specific methylation of PE-DHA (Vance, 1988; Samborski *et al.* 1993).

The major essential fatty acid for poultry is LA, and it is important for chick growth, egg production and egg size (Balnave, 1970; Watkins, 1991). Our results suggest that the *n*-3 series PUFA may also be important, as both EPA and DHA were incorporated to a significant extent into the breast-muscle phospholipids of chickens fed all dietary treatments. Interestingly, EPA and DHA were not present in the dietary fatty acid profile of either the sunflower oil or tallow diets. While these diets did contain a small concentration of  $\alpha$ -linolenic acid (18:3*n*-3), it comprised only 1.5% of the total dietary lipid. The EPA and DHA of the phospholipids from birds on these treatments was presumably synthesised from  $\alpha$ -linolenic acid through the sequence of alternating desaturation and chain-elongation reactions (Cook, 1991). The finding that both these fatty acid series were present in the membranes of all chickens regardless of the dietary fat type underscores their importance as an essential requirement for poultry diets.

In summary, we have shown that the *n*-3 and *n*-6 long-chain PUFA are preferentially assimilated into the membrane phospholipids of chicken breast muscle. Although dietary *n*-3 and *n*-6 PUFA induce similar physiological outcomes (Cunnane *et al.* 1986; Shimomura *et al.* 1990; Sanz *et al.* 2000; Newman *et al.* 2002), the present study indicates that this result is not reflected in the remodelling of the molecular species of the major phospholipid classes. Further studies are required to elucidate the mechanism(s) that alter energy metabolism initiated by these dietary fatty acids.

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