

## Dietary phosphatidylcholine impacts on growth performance and lipid metabolism in adult Genetically Improved Farmed Tilapia (GIFT) strain of Nile tilapia *Oreochromis niloticus*

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### Abstract

This study aimed to determine the effects of supplementing the diet of adult Nile tilapia *Oreochromis niloticus* with phosphatidylcholine (PC) on growth performance, body composition, fatty acid composition and gene expression. Genetically Improved Farmed Tilapia fish with an initial body weight of 83.1 (SD 2.9) g were divided into six groups. Each group was hand-fed a semi-purified diet containing 1.7 (control diet), 4.0, 6.5, 11.5, 21.3 or 41.0 g PC/kg diet for 68 d. Supplemental PC improved the feed efficiency rate, which was highest in the 11.5 g PC/kg diet. Weight gain and specific growth rate were unaffected. Dietary PC increased PC content in the liver and decreased crude fat content in the liver, viscera and body. SFA and MUFA increased and PUFA decreased in muscle with increasing dietary PC. *Cytoplasmic phospholipase A<sub>2</sub>* and *secreted phospholipase A<sub>2</sub>* mRNA expression were up-regulated in the brain and heart in PC-supplemented fish. PC reduced *fatty acid synthase* mRNA expression in the liver and visceral tissue but increased expression in muscle. Hormone-sensitive lipase and lipoprotein lipase expression increased in the liver with increasing dietary PC. *Growth hormone* mRNA expression was reduced in the brain and *insulin-like growth factor-1* mRNA expression in liver reduced with PC above 6.5 g/kg. Our results demonstrate that dietary supplementation with PC improves feed efficiency and reduces liver fat in adult Nile tilapia, without increasing weight gain, representing a novel dietary approach to reduce feed requirements and improve the health of Nile tilapia.

**Key words:** Phosphatidylcholine: Phospholipase A<sub>2</sub>: Lipid metabolic enzymes: Growth: Tilapia

The beneficial effects of dietary phospholipids (PL) on growth and survival have been demonstrated in the larval and juvenile stages of aquatic livestock species<sup>(1,2)</sup>. The beneficial effects of PL were not solely because of enhanced emulsification and digestion of lipids<sup>(3–5)</sup>, but also because of increased efficiency of dietary fatty acids and lipid transport from the gut to the rest of the body, potentially through enhanced lipoprotein synthesis<sup>(6–8)</sup>. Salmon and white sturgeon weighing more than 10 g were not reported to have a dietary requirement for PL, although this is potentially because of the short duration and limited assessment methods used in these preliminary studies<sup>(9,10)</sup>. To our knowledge, there have been no further studies investigating PL requirements in adult or individual fish weighing more than 50 g. Dietary PL has been shown to alleviate signs of liver disease in human and animal studies<sup>(11–13)</sup>. Meanwhile, the carbohydrate- and lipid-rich diets widely used in fish farming owing to shortages of protein resources commonly lead to the accumulation of ectopic fat, liver fat, mesenteric fat and also fat in the muscles of larger fish<sup>(14,15)</sup>.

Therefore, dietary supplementation with PL is potentially beneficial in the larger juvenile and adult fish that are increasingly affected by liver disease and metabolic disorders.

Previous research has commonly used crude mixed preparations of PL, particularly soyabean lecithin and other plant PL or egg-yolk lecithin. As these different sources are enriched in varying types of PL, it has been difficult to clarify which PL classes are responsible for the beneficial effects<sup>(2)</sup>. Phosphatidylcholine (PC), the most abundant class of PL in the diet, shows the greatest effect on fish larval performance<sup>(16)</sup>, with other studies reporting that supplementation with PC contributes to survival and reduces larval deformities<sup>(6–19)</sup>. The expression and regulation of key genes involved in lipid metabolism in response to dietary PC has not previously been studied in fish, particularly in adult fish.

Fish possesses a similar set of enzymes involved in lipid metabolism to mammals, including lipoprotein lipase (LPL), a key enzyme in lipid deposition and metabolism<sup>(20)</sup>, hormone-sensitive lipase (HSL), an enzyme involved in lipolysis<sup>(21)</sup>, fatty

**Abbreviations:** cPLA<sub>2</sub>, cytosolic phospholipase A<sub>2</sub>; FAS, fatty acid synthase; GH, growth hormone; HSL, hormone-sensitive lipase; IGF-1, insulin-like growth factor-1; LPL, lipoprotein lipase; PC, phosphatidylcholine; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; sPLA<sub>2</sub>, secretory phospholipase A<sub>2</sub>.

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acid synthase (FAS), involved in lipogenesis<sup>(22)</sup>, and phospholipase A<sub>2</sub> (PLA<sub>2</sub>), which catalyses the hydrolysis of membrane glycerol PL<sup>(23)</sup>. Previous research in Otsuka Long-Evans Tokushima fatty rats has shown that the effects of dietary PC were attributable to the suppression of FAS activity in the liver<sup>(24)</sup>. Injection of PC also increases HSL transcription in mouse fat tissue<sup>(25)</sup>, and has suggested that secreted PLA<sub>2</sub> has an important role in hepatic uptake and metabolism of PC<sup>(26)</sup>.

Tilapia is becoming one of the most important and fastest-growing fish species in aquaculture. The Genetically Improved Farmed Tilapia (GIFT) strain is a new nationally certificated strain selected over 14 years and nine generations from the base strain of Nile tilapia<sup>(27)</sup>. The GIFT strain is among the most successful of the introduced farmed tilapia in China owing to its strong adaptability, rapid growth, high fecundity and broad diet. On the basis of weight gain and feed efficiency, Kasper & Brown<sup>(5)</sup> concluded that PC is a beneficial nutrient for juvenile tilapia with an initial body weight of 12.4 g and recommended that purified diets fed to juveniles include 15 g PC/kg diet.

In the present study, we aimed to investigate the effects of varying levels of dietary PC on growth performance, tissue composition and the fatty acid profile in the tissues of adult GIFT strain Nile tilapia fish. In addition, we determined the effect of dietary PC on the expression of enzymes involved in lipid metabolism. We hypothesised that dietary PC has beneficial physiological effects in adult Nile tilapia, mediated through the altered expression of lipid enzymes.

## Methods

### Diets

Six semi-purified diets were prepared. Casein, gelatin and soyabean meal, which were the main protein sources, provided

30.6% dietary protein (the protein requirement for maximum growth performance of large tilapia is approximately 30%)<sup>(28)</sup>. Soyabean oil and PC were used as lipid sources; they provided 7.6% dietary lipid for large tilapia<sup>(29)</sup>. The diets were supplemented with different levels of dietary PC – 0, 2.5, 5.0, 10.0, 20.0 or 40.0 g/kg diet – and the dietary lipid levels were adjusted by the soyabean oil levels. The analysed level of PC in the six diets was 1.7 (the control group), 4.0, 6.5, 11.5, 21.3 and 41.0 g/kg diet. The diet preparation was conducted as previously described<sup>(29)</sup>. The ingredients and proximate composition and fatty acid profiles are shown in Tables 1 and 2.

### Experimental procedure

The feeding trial was performed in an indoor recirculating aquarium system at the Yangtze River Fisheries Research Institute (Wuhan, Hubei Province, China). GIFT strain fish were obtained from the Guangxi tilapia national breeding farm (Nanning, Guangxi Province, China), and were maintained in a concrete pool (3 × 3 × 5 m) at the experimental base. During the acclimatisation period, fish were fed the basal diet to adjust to the experimental diets and environmental conditions for 2 weeks.

During the initial phase, fish were fasted for 24 h and weighed after being anaesthetised with 80 mg/l MS-222. Adult male fish (initial weight: 83.12 (SD 2.84) g) were randomly assigned to eighteen tanks (500 litres) with twenty fish/tank. Three tanks of fish were randomly assigned to each diet. To reduce pellet waste, fish were gradually hand-fed until they appeared satiated by observing their feeding behaviour. The fish were fed three times a day: 08.30, 12.30 and 16.30 hours (natural photoperiod). The feeding trial lasted 68 d. During this period, food consumption and any fish deaths were recorded daily. The water was maintained at 28–34°C, pH of 7.4–7.6, with

**Table 1.** Composition and proximate analysis of the experimental diets

Ingredients	G0	G0.25	G0.5	G1	G2	G4
Casein	200	200	200	200	200	200
Gelatin	50	50	50	50	50	50
Soyabean meal	200	200	200	200	200	200
Soyabean oil	75	72.5	70	65	55	35
Phosphatidylcholine	0	2.5	5.0	10	20	40
Dextrin	340	340	340	340	340	340
Cellulose	71.5	71.5	71.5	71.5	71.5	71.5
Monocalcium phosphate	10	10	10	10	10	10
Vitamin premix*	10	10	10	10	10	10
Mineral premix†	40	40	40	40	40	40
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5
Butylated hydroxytoluene	1	1	1	1	1	1
Total	1000	1000	1000	1000	1000	1000
Proximate composition (g/kg diet)						
DM	909.9	915.5	913.6	913.5	913.7	913.9
Crude protein	307.6	305.6	306.5	306.2	306.6	305.8
Crude lipid	74.6	75.7	75.4	78.7	75.1	76.4
Ash	39.8	39.9	39.5	40.1	41.3	42.6
Phosphatidylcholine	1.7	4.0	6.5	11.5	21.3	41.0

\* Vitamin premix contained (g premix): thiamine hydrochloride, 5 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; nicotinic acid, 6.05 mg; L-ascorbyl-2-monophosphate-Mg, 3.95 mg; pyridoxine hydrochloride, 4 mg; folic acid, 1.5 mg; inositol, 200 mg; menadione, 4 mg; α-tocopheryl acetate, 50 mg; retinyl acetate, 60 mg; biotin, 0.6 mg. All ingredients were diluted with α-cellulose to 1 g.

† Mineral premix contained (kg diet): calcium biphosphate, 13.58 g; calcium lactate, 32.7 g; FeSO<sub>4</sub>·6H<sub>2</sub>O, 2.97 g; magnesium sulphate, 13.7 g; potassium phosphate dibasic, 23.98 g; sodium biphosphate, 8.72 g; sodium chloride, 4.35 g; AlCl<sub>3</sub>·6H<sub>2</sub>O, 0.015 g; KI, 0.015 g; CuCl<sub>2</sub>, 0.01 g; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.08 g; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.1 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.3 g.

**Table 2.** Fatty acid composition (% of total fatty acids) of the experimental diets

Fatty acids	G0	G0.25	G0.5	G1	G2	G4
<b>SFA</b>						
C14:0	0.74	0.76	0.76	0.78	0.73	0.78
C16:0	15.96	15.53	16.21	15.93	16.23	19.92
C18:0	2.78	2.32	2.14	2.60	2.74	3.26
<b>MUFA</b>						
C18:1	24.78	26.05	26.61	24.63	25.37	22.45
<i>n</i> -6 Fatty acids						
C18:2	50.25	49.52	48.75	50.49	49.23	48.25
<i>n</i> -3 Fatty acids						
C18:3	5.49	5.82	5.59	5.57	5.70	5.34

**Table 3.** Nucleotide sequences of primers and cycling conditions used for PCR amplification

Genes	Forward primer (5'–3')	Reverse primer (5'–3')	T <sub>m</sub> (°C)	Amplicon size (bp)
<i>FAS</i>	TGAAACTGAAGCCTTGTGTGCC	TCCCTGTGAGCGGAGGTGATTA	62	141
<i>LPL</i>	TGCTAATGTGATTGTGGTGGAC	GCTGATTTTGTGGTTGGTAAGG	59	217
<i>HSL</i>	GCCCTATCTAAGAGTTGGTCC	CGATGTCGTACACATAAGTTG	62	214
<i>GH</i>	ACAGCCAGCGTTTGTCTCCAT	GGAACCTCCAGGACTCAACCA	62	250
<i>IGF-1</i>	TGCGATGTGCTGTATCTCCTG	GCCATAGCCTGTTGGTTTATTG	60	178
<i>sPLA<sub>2</sub></i>	TACAAGCCAGTGCCTCGTCC	AGGTGTCATAGCAGCGGTCA	61	139
<i>cPLA<sub>2</sub></i>	ACCGTGAAGGTCTGAAGGAAT	CTCAGGGTTCGTCAAAAATGTC	59	182
<i>β-Actin</i>	TGGTGGGTATGGGTCAGAAAG	CTGTTGGCTTTGGGGTTCA	59–62	216

T<sub>m</sub>, melting temperature; *FAS*, fatty acid synthase; *LPL*, lipoprotein lipase; *HSL*, hormone-sensitive lipase; *GH*, growth hormone; *IGF-1*, insulin-like growth factor-1; *sPLA<sub>2</sub>*, secretory phospholipase A<sub>2</sub>; *cPLA<sub>2</sub>*, cytosolic phospholipase A<sub>2</sub>.

a dissolved O<sub>2</sub> concentration >6 mg/l. During the feeding trial, fish were weighed and counted every 2 weeks after 24 h of fasting for analysis of growth and feeding. All experiments were conducted using a protocol approved by the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

### Sample collection

At the end of the experiment, fish were weighed and counted following a 24-h fast. Eight fish were removed from each tank using a dipnet and sedated with 80 mg/l MS-222 and were designated as the sample fish. The body weight of each sample fish was similar to the average fish weight of each tank. Three sample fish from each tank were killed and the bodies, viscera and liver were collected and weighed to determine the viscerosomatic index (VSI) and the hepatosomatic index (HSI). The liver, viscera and dorsal muscle tissues were stored at –80°C until analysis of tissue composition and fatty acid analysis. A further two sample fish from each tank were killed for whole-body composition analyses. The whole body was first cut into small pieces with a knife, and then minced with a meat grinder; the minced meat was mixed thoroughly, and samples of approximately 100 g were taken from each fish and frozen at –80°C. The remaining three sample fish per tank were sedated with 80 mg/l MS-222, disinfected with 75% alcohol and killed. Approximately 0.2 g of brain, heart, liver, muscle and visceral tissue was placed in 2-ml microcentrifuge tubes, frozen in liquid N<sub>2</sub> and stored at –80°C until mRNA expression analyses.

### Biochemical analyses

Crude protein, crude fat and ash contents were measured using the Micro-Kjeldahl, Soxhlet and ignition methods,

respectively. Moisture content was determined using the freeze-drying method, in which samples were freeze-dried for 48 h in a vacuum freeze dryer (Christ Beta 2–4 LD plus LT; Marin Christ Corporation). Fatty acid content of diets and tissues was determined as previously described by Liu *et al.*<sup>(30)</sup>. Fatty acid methyl esters were separated, and quantified by GC-2010 gas chromatograph (SHIMADZU) with a fused silica capillary column (SP-2560; Supelco; 100 m × 0.32 mm i.d., film thickness 0.20 μm) and a flame ionisation detector (FID). The thermal gradient programme was initially 100°C for 3 min, followed by increments of 5°C/min and finally 250°C for 10 min. The injector and FID temperatures were 250°C. PC contents in samples of the diets, muscle and liver were determined at the China National Analytical Center. The quantitative determination of PC in samples was conducted using the HPLC – Refractive Index (RI) method. A Waters Spherisorb S5W column (4.6 × 250 mm packed with 5-μm silica) and an isocratic mobile phase consisting of *n*-hexane–2-propanol–water (1:4:1, by vol.) were used. The HPLC conditions were as follows: flow rate 0.8 ml/min, injection aliquot 10 μl, column temperature 35°C and temperature of the RI detector (differential refractive index detector) 35°C. The content of PC in samples was determined by the external standard method, using soyabean PC as the external standard.

The sequences of the primer pairs used for real-time PCR (RT-PCR) analysis of gene expression of secretory phospholipase A<sub>2</sub> (*sPLA<sub>2</sub>*), cytosolic phospholipase A<sub>2</sub> (*cPLA<sub>2</sub>*), *FAS*, *LPL*, *HSL*, growth hormone (*GH*), insulin-like growth factor-1 (*IGF-1*) and *β-actin* are shown in Table 3. *β-actin* was selected as the housekeeping gene for the normalisation of gene expression. RT-PCR reactions were conducted as previously described<sup>(29)</sup>.

**Statistical analyses**

The data were analysed using one-way ANOVA and Duncan's multiple-range tests using SPSS 17.0 for Windows (SPSS). Data are expressed as means and standard deviations in tables and figures. Differences were considered significant at  $P < 0.05$ .

**Results**

**Growth performance and physiological parameters**

The effects on growth performance and physiological parameters of feeding adult Nile tilapia diets containing increasing amounts of PC are shown in Table 4. Fish fed diets containing 4.0 to 21.3 g/kg of PC showed a higher feed efficiency rate ( $P < 0.05$ ) than those eating the control diet. The feeding rate was not significantly different among diet groups, ranging from 1.75 to 1.81%. The

weight gain and specific growth rate were lower in the G4 group than in the G0.5 group ( $P < 0.05$ ), but no other differences were found in weight gain and specific growth rate between fish fed diets with added PC or the control diet. Fish in group G1 showed the highest VSI and HSI. No fish died during the feeding trial.

**Whole body and tissue composition**

Table 5 shows the effects of varying levels of dietary PC on whole body and tissue composition. As the amount of dietary PC increased, whole body crude fat content decreased from 10.79 to 9.77% ( $P < 0.05$ ), liver crude fat content decreased from 10.53 to 8.34% ( $P < 0.05$ ) and visceral crude fat content decreased from 21.95 to 19.26% ( $P < 0.05$ ). The crude fat content in muscle increased from 1.68 to 2.34% ( $P < 0.05$ ) as dietary PC levels increased from 1.7 to 11.5 g/kg and then subsequently

**Table 4.** Growth performance and physiological parameters for Nile tilapia fed diets containing different phosphatidylcholine (PC) levels for 68 d (Mean values and standard deviations;  $n$  3)

Index	G0		G0.25		G0.5		G1		G2		G4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial weight (g)	83.44	2.40	81.07	3.58	82.96	3.58	84.89	4.73	81.44	2.95	85.60	1.15
Final weight (g)	268.52	10.66	264.64	9.89	270.86	2.60	277.38	18.91	265.22	12.53	267.51	1.63
Weight gain (%) <sup>*</sup>	225.29 <sup>a,b</sup>	5.02	226.60 <sup>a,b</sup>	8.85	233.66 <sup>a</sup>	4.67	226.59 <sup>a,b</sup>	4.22	225.60 <sup>a,b</sup>	5.48	212.55 <sup>b</sup>	4.81
Specific growth rate <sup>†</sup>	1.72 <sup>a,b</sup>	0.03	1.72 <sup>a,b</sup>	0.01	1.77 <sup>b</sup>	0.02	1.74 <sup>b</sup>	0.02	1.74 <sup>b</sup>	0.02	1.67 <sup>a</sup>	0.02
Feed efficiency rate (%) <sup>‡</sup>	82.95 <sup>a</sup>	1.45	88.18 <sup>b,c</sup>	0.53	88.68 <sup>c</sup>	1.19	94.86 <sup>d</sup>	2.82	88.58 <sup>c</sup>	0.43	85.16 <sup>a,b</sup>	1.27
Feeding rate (%) <sup>§</sup>	1.81	0.08	1.81	0.06	1.76	0.02	1.75	0.10	1.80	0.05	1.81	0.05
Hepatosomatic index (%) <sup>  </sup>	1.98 <sup>a</sup>	0.11	2.04 <sup>a</sup>	0.12	2.60 <sup>b</sup>	0.14	2.64 <sup>b</sup>	0.24	2.43 <sup>b</sup>	0.20	2.08 <sup>a</sup>	0.11
Viscerosomatic index (%) <sup>¶</sup>	9.80 <sup>a,b</sup>	0.87	9.77 <sup>a,b</sup>	0.63	10.12 <sup>a,b</sup>	0.78	10.50 <sup>b</sup>	0.63	9.71 <sup>a,b</sup>	0.98	9.33 <sup>a</sup>	0.42

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

<sup>\*</sup> Weight gain (WG, %) =  $100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$ .

<sup>†</sup> Specific growth ratio (SGR, %/d) =  $100 \times \ln(\text{final weight}/\text{initial weight}) / \text{d}$ .

<sup>‡</sup> Feed efficiency rate (FER, %) =  $100 \times \text{wet weight gain} / \text{dry feed consumed}$ .

<sup>§</sup> Feeding rate (FR) =  $100 \times \text{dry feed consumed} \times 2 / (\text{final body weight} + \text{initial body weight}) / \text{d}$ .

<sup>||</sup> Hepatosomatic index (HSI, %) =  $100 \times (\text{hepatosomatic weight} / \text{whole body weight})$ .

<sup>¶</sup> Viscerosomatic index (VSI, %) =  $100 \times (\text{viscera weight} / \text{whole body weight})$ .

**Table 5.** Proximate tissues and whole-body compositions of Nile tilapia fed diets containing different levels of phosphatidylcholine (PC) for 68 d (%) (wet mass) (Mean values and standard deviations;  $n$  3)

Index	G0		G0.25		G0.5		G1		G2		G4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Whole body</b>												
Moisture	67.83	0.22	67.88	0.70	68.23	0.51	68.63	0.37	68.40	0.86	68.66	0.84
Crude protein	16.32 <sup>a</sup>	0.47	16.21 <sup>a</sup>	0.36	16.31 <sup>a</sup>	0.54	15.41 <sup>b</sup>	0.36	15.93 <sup>a,b</sup>	0.33	16.06 <sup>a</sup>	0.38
Crude fat	10.79 <sup>a</sup>	0.43	10.02 <sup>b</sup>	0.51	10.22 <sup>b</sup>	0.41	10.14 <sup>b</sup>	0.43	9.76 <sup>b</sup>	0.32	9.77 <sup>b</sup>	0.42
Ash	3.94	0.21	3.43	0.18	3.42	0.34	3.74	0.08	3.52	0.43	3.81	0.27
<b>Muscle</b>												
Moisture	77.89	0.54	77.17	0.752	76.59	0.46	76.49	0.23	76.95	0.74	76.69	0.47
Crude protein	18.88 <sup>a</sup>	0.25	19.54 <sup>b</sup>	0.08	19.41 <sup>b</sup>	0.43	19.31 <sup>b</sup>	0.39	19.43 <sup>b</sup>	0.39	19.38 <sup>b</sup>	0.21
Crude fat	1.68 <sup>a</sup>	0.12	1.72 <sup>a</sup>	0.15	2.18 <sup>b,c</sup>	0.08	2.32 <sup>c</sup>	0.29	1.98 <sup>b</sup>	0.09	1.67 <sup>a</sup>	0.13
Ash	1.31	0.07	1.32	0.03	1.30	0.05	1.27	0.03	1.27	0.02	1.27	0.03
<b>Liver</b>												
Moisture	64.97 <sup>a</sup>	0.92	64.44 <sup>a</sup>	1.04	64.97 <sup>a</sup>	0.34	64.58 <sup>a</sup>	0.40	65.27 <sup>a</sup>	0.58	66.68 <sup>b</sup>	0.73
Crude protein	9.89	0.62	9.07	0.86	8.96	0.65	8.78	0.59	9.08	0.18	9.50	0.19
Crude fat	10.53 <sup>a</sup>	0.61	10.26 <sup>a,b</sup>	0.39	9.77 <sup>b,c</sup>	0.76	9.92 <sup>b,c</sup>	0.79	9.77 <sup>b,c</sup>	0.91	8.34 <sup>c</sup>	0.42
<b>Viscera</b>												
Moisture	61.25	0.92	59.84	1.08	59.51	1.11	60.79	0.72	60.12	1.01	61.34	2.13
Crude protein	8.71 <sup>a</sup>	0.28	8.70 <sup>a</sup>	0.73	8.29 <sup>a,b</sup>	0.35	8.30 <sup>a,b</sup>	0.71	8.06 <sup>a,b</sup>	0.51	7.73 <sup>b</sup>	0.53
Crude fat	21.95 <sup>a</sup>	1.06	21.22 <sup>a,b</sup>	0.45	21.50 <sup>a,b</sup>	1.28	19.85 <sup>b</sup>	1.09	19.51 <sup>b</sup>	0.85	19.26 <sup>b</sup>	1.14

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

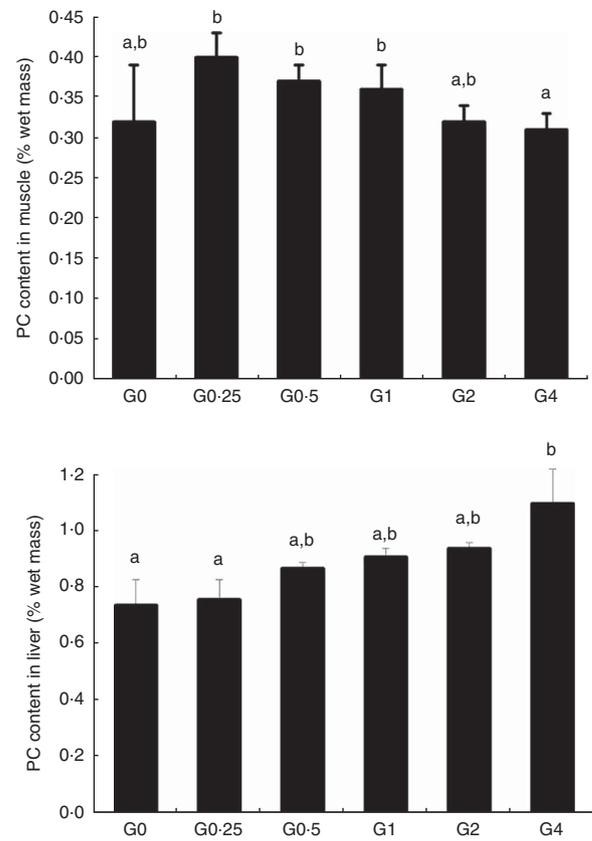
decreased to 1.67% when dietary PC increased further from 21.3 to 41 g/kg. Moisture concentrations in liver were significantly higher in the G4 group than in other groups ( $P < 0.05$ ). Moisture concentrations in muscle, viscera and whole body were unchanged with increasing dietary PC levels. The G1 group had the lowest protein content in the whole body. With increasing dietary PC level, crude protein content in muscle in PC-added groups significantly increased compared with fish fed the control diet ( $P < 0.05$ ). Liver protein content was not affected by dietary PC level. Increasing dietary PC levels resulted in crude protein content in viscera decreasing from 8.71 to 7.73% ( $P < 0.05$ ). No significant differences were found in the ash content of whole body or muscle.

### Phosphatidylcholine content of muscle and liver

Fig. 1 shows the PC content of muscle and liver in fish fed different dietary levels of PC. The PC content in the liver increased with greater amounts of PC in the diet, becoming significantly higher in the G4 group than in the control group ( $P < 0.05$ ). As dietary PC levels increased, the muscle content of PC in fish containing diets with added PC was not significantly different compared with that of the control group.

### Muscle and liver tissue fatty acid profile

In muscle, there were differences in total SFA, MUFA and PUFA in response to dietary PC level (Table 6). SFA and MUFA increased, whereas PUFA decreased with increasing dietary PC levels. In particular, C16:0 and C18:1 increased with increasing dietary PC levels, whereas linoleic acid (C18:2*n*-6, LA) was significantly



**Fig. 1.** Phosphatidylcholine (PC) content of muscle and liver in adult Nile tilapia fed diets containing different PC levels for 68 d. Values are means, and standard deviations represented by vertical bars. <sup>a,b</sup> Mean values with unlike letters were significantly different using Duncan's multiple-range test ( $P < 0.05$ ).

**Table 6.** Fatty acid composition (% of total fatty acids) of the muscle of Nile tilapia fed diets containing different phosphatidylcholine (PC) levels for 68 d (Mean values and standard deviations;  $n$  3)

Index	G0		G0.25		G0.5		G1		G2		G4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>SFA</b>												
C14:0	2.75 <sup>a</sup>	0.07	2.84 <sup>a</sup>	0.14	3.51 <sup>b</sup>	0.21	3.56 <sup>b</sup>	0.09	3.68 <sup>b</sup>	0.06	3.70 <sup>b</sup>	0.06
C16:0	19.96 <sup>a</sup>	1.09	22.28 <sup>c</sup>	0.82	20.19 <sup>a,b</sup>	0.89	22.04 <sup>b,c</sup>	0.27	22.94 <sup>c</sup>	0.31	23.91 <sup>c</sup>	0.52
C18:0	17.37 <sup>a</sup>	0.98	21.22 <sup>b</sup>	0.94	21.10 <sup>b</sup>	0.59	16.91 <sup>a</sup>	0.98	16.55 <sup>a</sup>	0.66	18.73 <sup>a</sup>	0.53
C20:0	1.41	0.04	1.51	0.05	1.53	0.06	1.51	0.02	1.50	0.18	1.43	0.06
<b>MUFA</b>												
C14:1	0.18	0.06	0.19	0.04	0.18	0.06	0.19	0.06	0.30	0.06	0.21	0.03
C16:1	5.71 <sup>a</sup>	0.37	5.78 <sup>a</sup>	0.23	6.20 <sup>a,b</sup>	0.33	6.88 <sup>b</sup>	0.42	6.97 <sup>b</sup>	0.25	6.69 <sup>b</sup>	0.18
C18:1	26.81 <sup>a</sup>	0.71	26.73 <sup>a</sup>	0.53	26.08 <sup>a</sup>	0.25	30.31 <sup>b</sup>	0.55	31.11 <sup>b</sup>	0.83	29.56 <sup>b</sup>	0.69
<b><i>n</i>-6</b>												
C18:2	16.32 <sup>e</sup>	0.55	12.11 <sup>c,d</sup>	0.68	13.57 <sup>d</sup>	0.70	11.41 <sup>b,c</sup>	0.81	10.23 <sup>a,b</sup>	0.38	9.39 <sup>a</sup>	0.66
C18:3	1.06 <sup>a</sup>	0.09	0.80 <sup>b</sup>	0.04	0.92 <sup>a,b</sup>	0.04	0.86 <sup>a,b</sup>	0.13	0.73 <sup>b,c</sup>	0.09	0.64 <sup>c</sup>	0.09
C20:4	1.47	0.20	1.70	0.15	1.69	0.12	1.37	0.09	1.37	0.13	1.43	0.10
<b><i>n</i>-3</b>												
C18:3	1.07 <sup>a</sup>	0.05	0.81 <sup>b</sup>	0.06	0.84 <sup>b</sup>	0.04	0.76 <sup>b,c</sup>	0.04	0.64 <sup>c</sup>	0.04	0.51 <sup>d</sup>	0.04
C18:4	0.15	0.02	0.11	0.02	0.10	0.02	0.13	0.02	0.12	0.02	0.11	0.01
C20:5	1.21 <sup>a</sup>	0.03	1.04 <sup>a</sup>	0.15	0.95 <sup>a,b</sup>	0.05	0.91 <sup>b</sup>	0.08	0.89 <sup>b</sup>	0.04	0.98 <sup>b</sup>	0.10
C22:6	0.57	0.02	0.59	0.02	0.51	0.05	0.52	0.08	0.45	0.08	0.50	0.07
Total SFA	41.49 <sup>a</sup>	0.51	47.86 <sup>c</sup>	0.45	46.34 <sup>b,c</sup>	0.62	44.02 <sup>b</sup>	1.16	44.68 <sup>b</sup>	1.05	47.78 <sup>c</sup>	1.07
Total MUFA	32.70 <sup>a</sup>	1.05	32.70 <sup>a</sup>	0.37	32.46 <sup>a</sup>	0.38	37.38 <sup>b</sup>	0.75	38.39 <sup>b</sup>	0.63	36.46 <sup>b</sup>	0.82
Total PUFA	21.85 <sup>e</sup>	0.62	17.15 <sup>c,d</sup>	0.48	18.59 <sup>d</sup>	0.74	15.96 <sup>b,c</sup>	0.87	14.43 <sup>a,b</sup>	0.59	13.56 <sup>a</sup>	0.70
Total <i>n</i> -6	18.85 <sup>e</sup>	0.60	14.62 <sup>c,d</sup>	0.53	16.18 <sup>d</sup>	0.64	13.64 <sup>b,c</sup>	0.85	12.33 <sup>a,b</sup>	0.60	11.46 <sup>a</sup>	0.62
Total <i>n</i> -3	3.00 <sup>c</sup>	0.07	2.53 <sup>b</sup>	0.09	2.40 <sup>a,b</sup>	0.13	2.33 <sup>a,b</sup>	0.02	2.11 <sup>a</sup>	0.16	2.11 <sup>a</sup>	0.22

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

decreased in muscle from the higher PC groups, with the G4 group containing 57% less LA than the control group ( $P < 0.05$ ).

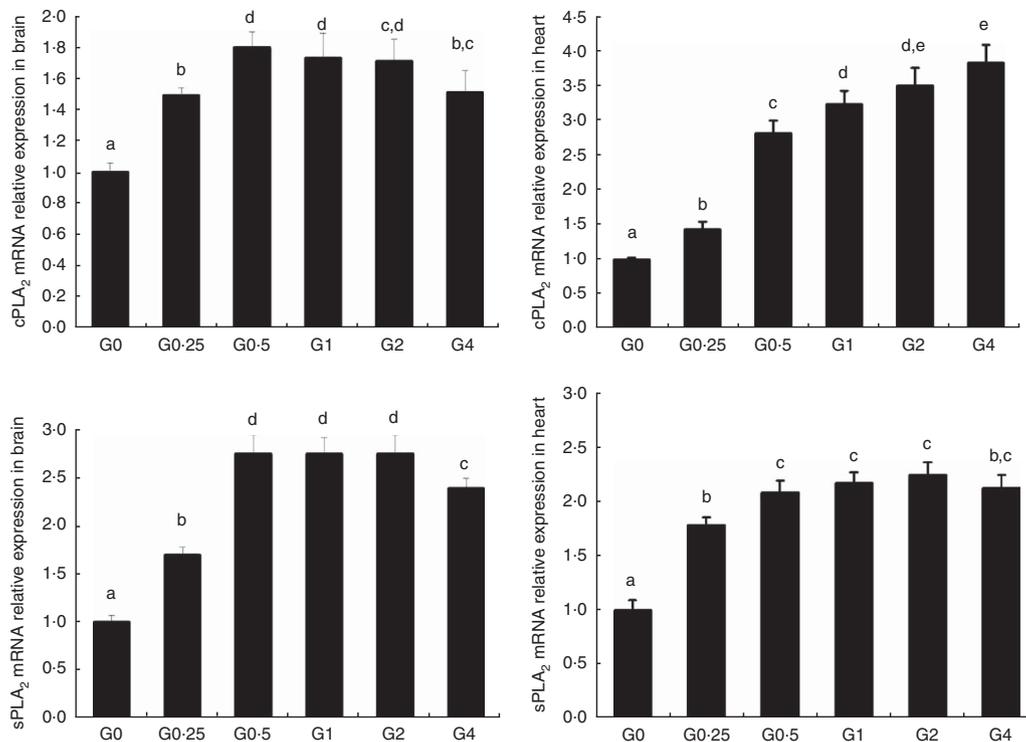
SFA in the liver decreased from 29.27 to 27.88% ( $P < 0.05$ ) as dietary PC levels increased from 1.7 to 6.5 g/kg, and then

increased to 31.53% as dietary PC increased to 41 g/kg. No significant differences were obtained in MUFA and PUFA content in fish fed additional PC compared with the control group (Table 7).

**Table 7.** Fatty acid composition (% of total fatty acids) of the liver of Nile tilapia fed diets containing different phosphatidylcholine (PC) levels for 68 d (%) (Mean values and standard deviations;  $n$  3)

Index	G0		G0.25		G0.5		G1		G2		G4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>SFA</b>												
C14:0	1.93 <sup>a</sup>	0.07	1.99 <sup>a,b</sup>	0.03	2.19 <sup>a,b,c</sup>	0.19	2.17 <sup>a,b,c</sup>	0.12	2.28 <sup>b,c</sup>	0.12	2.44 <sup>c</sup>	0.10
C16:0	20.42 <sup>a,b</sup>	0.04	19.81 <sup>a</sup>	0.21	19.59 <sup>a</sup>	0.74	20.23 <sup>a,b</sup>	0.30	21.37 <sup>b</sup>	0.37	22.67 <sup>c</sup>	0.51
C18:0	5.53	0.34	5.22	0.41	4.69	0.38	4.89	0.10	5.11	0.29	5.16	0.29
C20:0	1.39 <sup>b</sup>	0.06	1.18 <sup>a</sup>	0.02	1.41 <sup>b</sup>	0.10	1.21 <sup>a</sup>	0.07	1.39 <sup>b</sup>	0.03	1.26 <sup>a,b</sup>	0.05
<b>MUFA</b>												
C14:1	0.26	0.06	0.28	0.08	0.23	0.02	0.22	0.02	0.21	0.03	0.18	0.03
C16:1	3.37 <sup>a</sup>	0.19	3.46 <sup>a</sup>	0.13	3.74 <sup>a,b</sup>	0.16	4.04 <sup>b,c</sup>	0.14	4.09 <sup>b,c</sup>	0.07	4.32 <sup>c</sup>	0.18
C18:1	30.98	0.88	31.37	0.64	31.23	1.70	30.97	1.10	30.29	1.02	30.62	0.25
<b>n-6</b>												
C18:2	23.30 <sup>b</sup>	0.46	24.19 <sup>b</sup>	0.83	23.83 <sup>b</sup>	1.27	22.39 <sup>a,b</sup>	0.12	23.14 <sup>b</sup>	0.31	20.59 <sup>a</sup>	0.76
C18:3	1.05 <sup>a,b</sup>	0.05	1.31 <sup>c</sup>	0.12	1.22 <sup>b,c</sup>	0.08	1.21 <sup>b,c</sup>	0.03	1.04 <sup>a,b</sup>	0.08	0.88 <sup>a</sup>	0.02
C20:4	3.00 <sup>b</sup>	0.20	2.50 <sup>a</sup>	0.22	2.82 <sup>a,b</sup>	0.26	3.22 <sup>b</sup>	0.16	2.89 <sup>a</sup>	0.12	3.06 <sup>a,b</sup>	0.15
<b>n-3</b>												
C18:3	1.49 <sup>b,c</sup>	0.12	1.84 <sup>d</sup>	0.04	1.71 <sup>c,d</sup>	0.14	1.56 <sup>b,c</sup>	0.04	1.46 <sup>b</sup>	0.01	1.22 <sup>a</sup>	0.03
C20:5	2.18 <sup>b</sup>	0.15	1.78 <sup>a</sup>	0.03	1.85 <sup>a,b</sup>	0.12	2.07 <sup>a,b</sup>	0.14	1.99 <sup>a,b</sup>	0.09	2.14 <sup>b</sup>	0.19
C22:6	1.20	0.06	1.00	0.08	1.11	0.10	1.19	0.07	1.10	0.17	1.18	0.03
Total SFA	29.27 <sup>b,c</sup>	0.44	28.21 <sup>a,b</sup>	0.64	27.88 <sup>a</sup>	0.61	28.51 <sup>a,b</sup>	0.20	30.13 <sup>c</sup>	0.40	31.53 <sup>d</sup>	0.53
Total MUFA	34.51	0.96	35.11	0.55	35.21	1.67	35.22	1.09	34.49	0.96	35.09	0.06
Total PUFA	32.23 <sup>a,b</sup>	0.62	32.23 <sup>a,b</sup>	1.19	32.55 <sup>b</sup>	1.62	31.63 <sup>a,b</sup>	0.30	31.63 <sup>a,b</sup>	0.69	29.06 <sup>a</sup>	0.93
Total n-6	27.36 <sup>b</sup>	0.64	28.01 <sup>b</sup>	1.10	27.88 <sup>b</sup>	1.49	26.81 <sup>a,b</sup>	0.24	27.08 <sup>b</sup>	0.50	24.53 <sup>a</sup>	0.90
Total n-3	4.87	0.12	4.63	0.09	4.67	0.13	4.82	0.20	4.55	0.19	4.53	0.20

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



**Fig. 2.** The effects of dietary phosphatidylcholine (PC) levels on the mRNA expression levels of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) in brain and heart for adult Nile tilapia. Values are means, and standard deviations represented by vertical bars. cPLA<sub>2</sub>, cytosolic phospholipase A<sub>2</sub>; sPLA<sub>2</sub>, secretory phospholipase A<sub>2</sub>. a,b,c,d,e Mean values with unlike letters were significantly different using Duncan's multiple-range test ( $P < 0.05$ ).

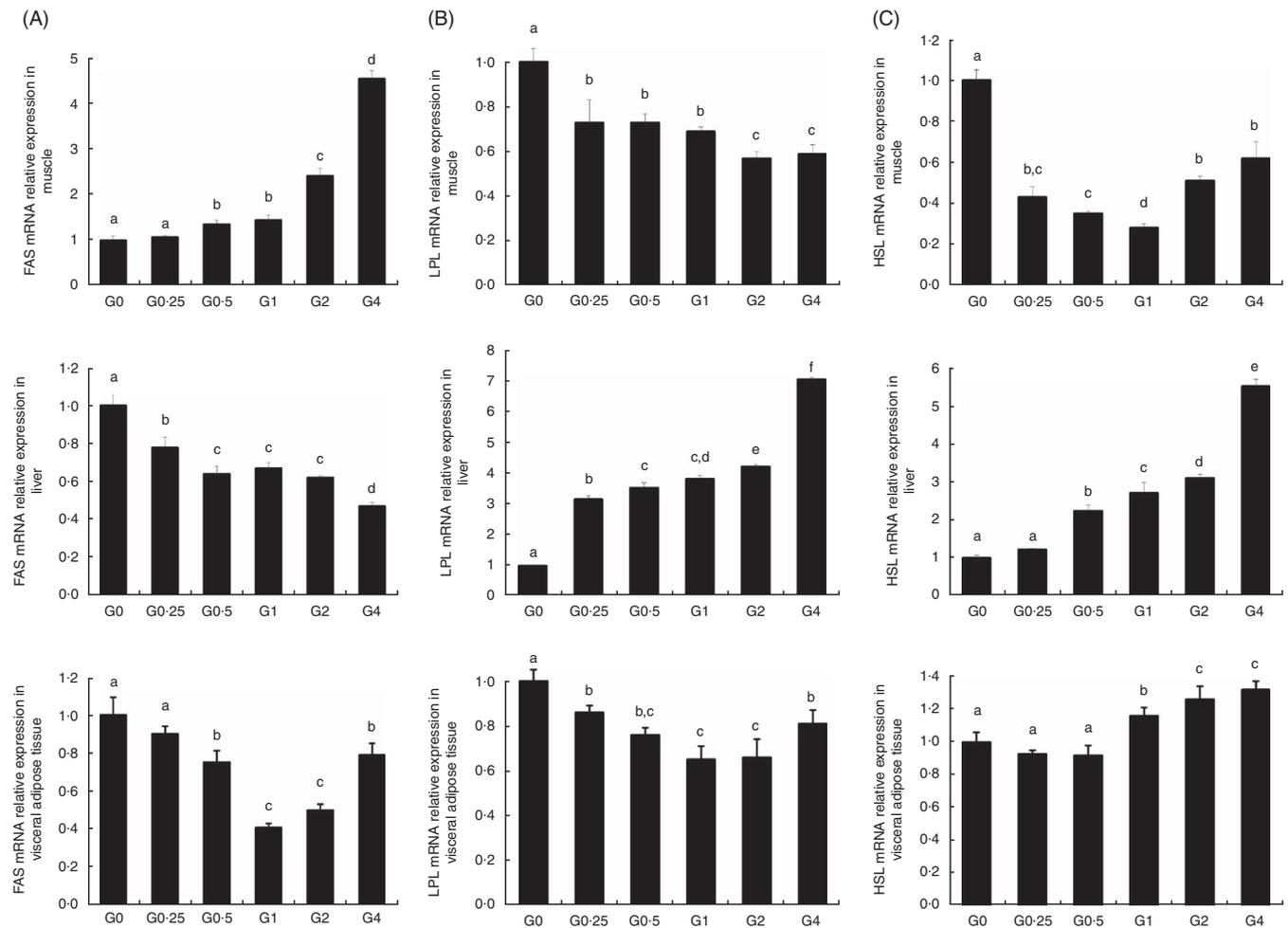
*Relative mRNA expression levels*

The expression of *cPLA<sub>2</sub>* and *sPLA<sub>2</sub>* mRNA was up-regulated in brain and heart with increased dietary PC levels compared with the control diet group without added PC (Fig. 2). Increased dietary PC reduced *FAS* mRNA expression in the liver and *FAS* mRNA expression in visceral tissue was reduced from the G0.5 to the G4 group. In contrast, *FAS* mRNA expression increased in the muscle from the G0.5 to the G4 group (Fig. 3(A)). In the PC-fed groups, there was a significant up-regulation in *LPL* mRNA expression in liver and visceral tissue ( $P < 0.05$ ), and down-regulation in muscle ( $P < 0.05$ ) (Fig. 3(B)). Compared with the control group, the PC-fed groups showed higher *HSL* mRNA levels in the liver (from the G0.5 to the G4 group) and visceral tissue (from the G1 to the G4 group). However, *HSL* mRNA levels in muscle were significantly lower in the PC-fed fish than in the control group ( $P < 0.05$ ) (Fig. 3(C)). *GH* mRNA in brain was significantly down-regulated with PC levels of 6.5 g/kg or above ( $P < 0.05$ ), whereas *IGF-1* mRNA in liver was significantly down-regulated at a PC level above 6.5 g/kg ( $P < 0.05$ ) (Fig. 4).

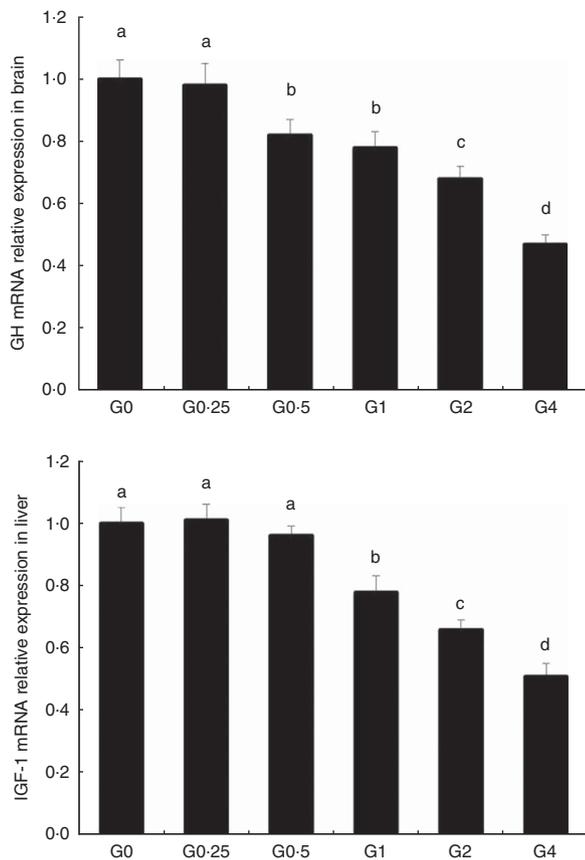
**Discussion**

The weight gain and specific growth rate for adult Nile tilapia remained largely unchanged by the addition of PC to the diet. This suggests that adult tilapia do not require additional PC for normal growth performance, which is consistent with previous results in juvenile large yellow croaker<sup>(31)</sup>, Atlantic salmon<sup>(10)</sup> and white sturgeon<sup>(4)</sup>. We measured the mRNA expression of *GH* in the brain and expression of *IGF-1* in liver, as the *GH/IGF* axis senses nutritional status and regulates body growth<sup>(32)</sup>, and *GH* and *IGF-1* mRNA are abundantly expressed in brain<sup>(33)</sup> and liver<sup>(34)</sup> in tilapia. The expression of *GH* mRNA in the brain and *IGF-1* mRNA in liver were down-regulated with higher levels of PC in the diet. This indicates that high levels of dietary PC reduce *GH* and *IGF* expression, potentially inhibiting the secretion of *GH*, and resulting in relatively slow growth performance.

As dietary PC levels increased, the crude fat content decreased in the whole body, liver and viscera, whereas PC content increased, indicating that dietary PC can reduce lipid accumulation in adult tilapia by regulating lipid metabolism. This is in agreement with previous results in large yellow croaker<sup>(31,35)</sup> and



**Fig. 3.** The effects of dietary phosphatidylcholine (PC) levels on the mRNA expression levels of genes involved in lipid metabolism in adult Nile tilapia for 68 d. (A) Fatty acid synthase (*FAS*) mRNA relative expression in liver, muscle and visceral adipose tissues. (B) Lipoprotein lipase (*LPL*) mRNA expression in liver, muscle and visceral adipose tissues. (C) Hormone-sensitive lipase (*HSL*) mRNA expression in liver, muscle and visceral adipose tissues. Values are means, and standard deviations represented by vertical bars. <sup>a,b,c,d,e,f</sup> Mean values with unlike letters were significantly different using Duncan's multiple-range test ( $P < 0.05$ ).



**Fig. 4.** The effects of dietary phosphatidylcholine (PC) levels on the mRNA expression levels of genes involved in growth hormone (*GH*) and insulin-like growth factor-1 (*IGF-1*) in adult Nile tilapia for 68 d. Values are means, and standard deviations represented by vertical bars. <sup>a,b,c,d</sup> Mean values with unlike letters were significantly different using Duncan's multiple-range test ( $P < 0.05$ ).

Dojo loach<sup>(36)</sup>, which suggest that PL may prevent and alleviate signs of liver disease in fish. We hypothesised that for fish fed diets with PC, the expression of genes related to lipogenesis would be lower, and the expression of genes related to lipolysis would be higher, ultimately resulting in lower lipid content. We investigated the effect of increased dietary PC on the expression of lipogenesis-related genes. PC increased the expression of LPL and HSL, enzymes involved in lipolysis, and decreased the expression of FAS, a lipogenic enzyme, in the liver. The level of LPL expression in a given tissue is the rate-limiting process for the uptake of TAG-derived FA<sup>(37)</sup>. LPL activity has previously been reported to be higher in the liver of cobia supplemented with PL<sup>(38)</sup>, and expression of LPL mRNA in the whole larval body significantly increased and expression of FAS mRNA decreased with the increasing levels of dietary PL in large yellow croaker larvae<sup>(39)</sup>. The expression of hepatic FAS mRNA was also decreased by diets with soyabean PL or EPA (EPA)-enriched PL in mice<sup>(12)</sup>. Our results confirm these previous findings, suggesting that PC increases the capacity to mobilise fat stores via regulation of competing lipolysis and lipogenesis enzymes, and these are consistent with the reduced lipid accumulation in the livers of Nile tilapia supplemented with PC. Unexpectedly, the higher dietary PC reduced expression of LPL and HSL in muscle, suggesting increased lipid deposition in muscle.

The fatty acid profiles in muscle and liver were affected by increasing PC levels, with SFA and MUFA increased and PUFA decreased in muscle. This may be related to variations in the fatty acid composition of the diets. Similarly, in Dojo loach, concentrations of total *n-3* fatty acids in the whole body significantly decreased with incremental dietary PL levels<sup>(36)</sup>. In contrast, rainbow trout fry fed egg lecithin containing high levels of EPA and DHA showed higher amounts of PUFA than soyabean lecithin and soyabean oil control groups<sup>(40)</sup>. Fatty acids are released from membrane PL by the action of PLA<sub>2</sub>, of which two main types are present. cPLA<sub>2</sub> are soluble in the cytosol and must first interact with or penetrate the organised lipid interface. sPLA<sub>2</sub> are linked to the cell membrane and, as a consequence, are unlikely to be structured such that penetration of the lipid interface is physiologically relevant<sup>(23)</sup>; sPLA<sub>2</sub> is highly expressed in the pancreas, brain, heart and liver tissues<sup>(41)</sup>. Nalefski *et al.*<sup>(42)</sup> first cloned the complementary DNA sequence of cPLA<sub>2</sub> from fish, and the cPLA<sub>2</sub> protein from the zebrafish shares a low (65%) similarity with that from humans. The activity of sPLA<sub>2</sub> in Atlantic cod was higher in liver, brain, kidney and gills than in muscle, potentially because of the regulatory roles that PLA<sub>2</sub> are thought to play in the brain and heart<sup>(43)</sup>. The expression of sPLA<sub>2</sub> mRNA in the whole body was positively correlated with dietary PL content in sea bass larvae<sup>(44)</sup>, and sPLA<sub>2</sub> activity in red drum *Sciaenops ocellatus* larvae also increased with increasing dietary PL<sup>(45)</sup>. In this study, we noted an up-regulation of cPLA<sub>2</sub> and sPLA<sub>2</sub> mRNA expression with increasing dietary PC levels in the brain and heart, suggesting that PLA<sub>2</sub> are potentially regulated via a positive feedback mechanism regulating the use of dietary PL in some fish.

### Conclusions

Moderate supplementation of the diet with PC was beneficial for improving feed efficiency and reducing liver fat for adult Nile tilapia, with no effect on weight gain. Excessive dietary PC may alter the expression of *LPL*, *HSL* and *FAS* mRNA in liver. Dietary supplementation with PC represents a potential new dietary approach to reduce feed requirements and improve the health of adult Nile tilapia in commercial aquaculture.

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H. W. and J. T. designed the research; J. T., L.-J. Y., X. L., W. L., F. W. and C.-G. Y. conducted the experiments and analysed the data; J. T. and M. J. wrote the paper. All authors have read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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