Growth and metabolic responses in Nile tilapia (*Oreochromis niloticus*) subjected to varied starch and protein levels of diets

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**ABSTRACT**

In the present study, six diets were formulated to contain 24% or 36% protein, and 0, 20%, or 40% starch. The Nile tilapias (*Oreochromis niloticus*) were fed with the designed diets for 56 days. An overall increasing tendency of percent weight gain, feed efficiency, protein efficiency rate (PER), hepatosomatic index (HSI), and condition factor (CF), whole-body protein and lipid contents, plasma concentrations of triglyceride (TG), total cholesterol (T-CHO) and glucose, liver glycogen contents, liver activities of glucokinase, pyruvate kinase, fatty acid synthetase, lipoprotein lipase (LPL), glucose-6-phosphate dehydrogenase, and malic enzyme with increasing dietary starch level was noted. While the opposite trend was found for feed intake (FI), whole-body moisture and ash contents, liver activities of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). Furthermore, all the parameters above were higher in fish fed the 36% vs. 24% protein diets, expect FI, PER, whole-body moisture, liver G6Pase and PEPCK were opposite, and furthermore HSI, whole-body lipid, liver glycogen and LPL were unaffected by dietary protein treatments. The results indicate that Nile tilapia appears to have a more sensitive response to dietary starch level than dietary protein level. Appropriate dietary starch supplementation could help promote the growth of the fish species. Low-dietary protein (24%) allowance may acquire growth comparable to that of Nile tilapia fed 36% protein diets when feed protein is provided only by fish meal with at least 20% starch supplementation.

**ARTICLE HISTORY**

Received 12 August 2016
Revised 15 December 2016
Accepted 20 December 2016

**KEYWORDS**

Carbohydrate; fish meal; nutrient utilisation; intermediary metabolism

**Introduction**

Fish need higher percentage and quality of dietary protein for growth than terrestrial animals (Wilson 1994). Fish meal is one of the most important and commonly used protein sources in aquafeed due to its high-protein quality with a balanced amino acids profile and good palatability. However, the stable supply of fish meal is usually limited and the need for aquafeed production is not being met, causing high cost of fish meal. In addition, protein is generally the most expensive of nutritional components in aquaculture, the biggest challenge in relation to the formulation of rations for fish is to meet the minimum requirement of protein for maximum growth while maintaining the appropriate balance of other components to meet their energy requirements. Part of challenges to be solved by the research on fish nutrition is to find mechanisms to diminish protein levels in diets by replacing them with cheaper non-protein energy sources like carbohydrates and lipids (Gao et al. 2011). Although finfish do not require carbohydrates in their diet, absence of carbohydrates in diet significantly reduces growth and daily weight gain due to muscle mass loss (Peragón et al. 1999) and the incorporation of complex carbohydrates in fish feeding has also been identified to decrease ammonia excretion, improve fish growth and spare protein in many fish species (Shiau & Cheng 1999; Peres & Oliva-Teles 2002; Wang et al. 2005; Wu et al. 2007). The ability of fish to use dietary carbohydrates differs among species (NRC 2011); diets with high levels of carbohydrates in carnivorous fish diminish consumption of food and their growth (Hemre et al. 2002), whereas omnivorous fish are more tolerant to carbohydrates using them more efficiently as energy source and even storing them as lipids (Shiau 1997). Therefore, it is of vital importance to evaluate nutritional value of carbohydrate and determine appropriate inclusion for feed formulations of cultured fish.
Tilapia is an omnivorous fish species that is cultured worldwide. The protein requirement of tilapia decreases with age and size (Siddiqui et al. 1988). In a study by Abdel-Tawwab et al. (2010), the optimum growth of fry (0.4–0.5 g) tilapia was obtained at 45% CP, while fingerling (17–22 g) and advanced juvenile (37–43 g) showed optimum growth performance with the 35% CP diet. Furthermore, protein requirement for optimum growth depends on dietary protein quality/source of the diets as well (NRC 2011). In this case, resembling the typical formulation employed in the literature (Ali et al. 2008; FIS (Fishery Industry Standards) 2005; Mjoun et al. 2010; NRC 2011), three starch levels (0, 20, and 40%) associated with two protein levels (24% and 36%) were utilised in this study to (1) investigate the interactive effects of protein and starch on the growth performance, plasma components, liver composition, and hepatic metabolic enzymes activities in Nile tilapia (Oreochromis niloticus); (2) determine whether adequate or insufficient dietary protein allowance affects the growth of tilapia when fish meal is provided as the only dietary protein source. Our objective was to gain insight into how Nile tilapias adjust growth and intermediary metabolism in response to both dietary starch and protein levels. The results of this study may be applicable in the practical tilapia aquaculture.

### Materials and methods

#### Experimental diets

Steam-dried fish meal containing 67.3% crude protein was used as the sole protein source. The profile of essential amino acids in fish meal was 3.94%, 1.96%, 2.83%, 4.73%, 6.22%, 2.08%, 2.90%, 2.86%, 0.62% and 3.22% for arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, respectively. Corn starch was used as the sole carbohydrate source. Six experimental diets were formulated to contain 24% or 36% protein and 0, 20% or 40% starch. The ingredients and proximate composition of the experimental diets are presented in Table 1. Microcrystalline cellulose and zeolite powder as filling ingredients were used to substitute starch and fish meal so as to obtain tested diets with fixed protein and lipid levels but varied starch levels. Doing so is to eliminate the interference of lipid on protein and starch utilisation in the determination of the interactive effects of dietary protein and starch on fish. All coarse dry ingredients were finely ground in a hammer mill and passed through a 60-mesh sieve, weighed, and blended together into a homogeneous mixture. All ingredients of each diet were blended until they were mixed uniformly. Water was added (approximately 50% of the diet weight) to the mixture and Table 1 was prepared accordingly.

#### Table 1. Ingredients and composition of the experimental diets (on an as-fed basis).

| Ingredients, % | Diet 1 (24P/0C) | Diet 2 (24P/20C) | Diet 3 (24P/40C) | Diet 4 (36P/0C) | Diet 5 (36P/20C) | Diet 6 (36P/40C) |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Fish meal*     | 35.82          | 35.82          | 35.82          | 53.73          | 53.73          | 53.73          |
| Maize starchb  | 0.00           | 20.00          | 40.00          | 0.00           | 20.00          | 40.00          |
| Microcrystalline cellulosec | 20.00          | 20.00          | 17.00          | 20.00          | 20.00          | 20.00          |
| Zeolite powderc | 37.00          | 17.00          | 0.00           | 20.25          | 0.25           | 0.00           |
| Fish oil       | 1.33           | 1.33           | 1.33           | 0.75           | 0.75           | 0.75           |
| Soy oil        | 1.33           | 1.33           | 1.33           | 0.75           | 0.75           | 0.75           |
| Soy lecithin   | 1.00           | 1.00           | 1.00           | 1.00           | 1.00           | 1.00           |
| Vitamin premixd | 0.20           | 0.20           | 0.20           | 0.20           | 0.20           | 0.20           |
| Mineral premixe | 0.20           | 0.20           | 0.20           | 0.20           | 0.20           | 0.20           |
| Ca(H2PO4)2      | 1.50           | 1.50           | 1.50           | 1.50           | 1.50           | 1.50           |
| Choline chloride | 0.10           | 0.10           | 0.10           | 0.10           | 0.10           | 0.10           |
| Vitamin C      | 0.02           | 0.02           | 0.02           | 0.02           | 0.02           | 0.02           |
| Sodium alginate | 1.50           | 1.50           | 1.50           | 1.50           | 1.50           | 1.50           |
| Nutrient level, % |               |               |               |               |               |               |
| Dry matter     | 91.76          | 91.80          | 91.96          | 90.75          | 90.86          | 90.78          |
| Crude protein  | 24.26          | 24.30          | 24.28          | 36.14          | 36.07          | 36.15          |
| Crude lipid    | 6.83           | 6.96           | 6.82           | 6.78           | 6.83           | 6.84           |
| Nitrogen free extract | 0.25          | 19.86          | 39.65          | 0.33           | 19.77          | 40.04          |
| Ash            | 40.13          | 23.83          | 6.61           | 29.63          | 9.75           | 8.89           |
| Available energyf | 8.47           | 11.90          | 15.24          | 11.26          | 14.61          | 18.12          |

*Fish meal was obtained from Baisuihang Feed Co. Ltd., Xiamen, China.

**Corn starch was obtained from Ganzhiyuan Sugar Co. Ltd., Nanjing, China.

*Microcrystalline cellulose and zeolite powder were obtained from Baisuihang Feed Co. Ltd., Xiamen, China.

**Vitamin premix (per kg diet): vitamin C (as Stay-C 35%) 400 mg; vitamin A (as vitamin A palmitate) 15,000 IU; vitamin D3 7500 IU; vitamin E (as D, L-a-tocopherol acetate) 150 mg; vitamin K3 (as menadione sodium bisulphite) 7.5 mg; vitamin B1 (as thiamine hydrochloride) 75 mg; riboflavin 60 mg; vitamin B2 (as pyridoxine hydrochloride) 100 mg; p-calcium pantothenate 120 mg; nicotinic acid 150 mg; biotin 1.0 mg; inositol 450 mg.

**Mineral premix (per kg diet): Fe (from Ferrous sulfate) 80 mg; Zn (from Zinc sulfate) 40 mg; Cu (from Copper sulfate) 5 mg; Mn (from Manganese sulfate) 67.3 mg; Se (from Sodium selenite) 0.2 mg; I (from Potassium iodine) 30 μg; Co (from Cobalt chloride) 50 μg; Mg (from Magnesium sulphate) 300 mg; P (from Sodium dihydrogen phosphate) 600 mg; K (from Potassium chloride) 740 mg.

fThe dietary available energy contents were calculated based on 23.6 KJ/g protein, 39.5 KJ/g lipid, and 17.2 KJ/g carbohydrate. Cellulose energy was excluded from the calculation in that microcrystalline cellulose is not utilised by fish.
mixed. The dough was extruded through a 2.5-mm die using multifunctional spiral extrusion machinery (CD4XITS; South China University of Technology, Guangzhou, China). The pellets were dried in a forced-air oven at 50 °C for 24 h and placed at room temperature for 24 h, and then sealed in plastic bags and kept at −20 °C until further use for feeding.

**Feeding procedures**

The tilapias obtained from a local commercial farm in Xiamen were transported to the aquaculture laboratory of Jimei University (Xiamen, China). The fish were stocked into a recirculating system consisting of two circular fibre glass tanks (0.85 m height × 1.22 m upper diameter, 1.04 m lower diameter) with a PolyGeyser® bead filter (Aquaculture Systems Technologies, LLC, New Orleans, Louisiana) and were initially maintained in a commercial tilapia feed for 15 days. Fish initially weighing an average of 10.29 ± 0.01 g (means ± standard deviation [SD]) were randomly categorised into 18 tanks at a density of 20 fish per tank (150 L). The fish in each group were hand fed with one diet to satiation at 08:00 and 18:00 h per day under natural photoperiod. Excess feed was collected 1 h after feeding. Growth trial lasted 6 weeks. Dissolved oxygen was measured daily and nitrite nitrogen (nitrite-N) was monitored twice a week using test kits (BioSino Bio-technology and Science, Inc., Beijing, China). Hepatic glycogen content was determined as described in Murat and Serfaty (1974). Lipid content of the liver was determined gravimetrically via chloroform-methanol extraction (Wang et al. 1993).

**Sampling procedures**

At the end of feeding trial, all fish in each tank were bulk weighed. Six fish from each tank were randomly sampled, anaesthetised using MS 222 solution (100 mg/L) and weighed individually. Blood samples were then collected via venipuncture using a heparinised syringe and immediately centrifuged at 3000 × g for 10 min at 4 °C. Then, plasma samples were stored at −80 °C for assay of blood biochemical parameters. After collection of blood, liver was removed and weighed. Liver was quick frozen in liquid nitrogen and stored at −80 °C until further analysis. Another five fish were randomly sampled and stored at −20 °C for determining whole-body composition.

**Proximate composition analysis**

Moisture, crude protein, crude lipid, and ash in the samples were determined according to the Association of Official Chemists (1995). Moisture content was determined using the drying method, using an oven at 105 °C. Crude protein content was determined by the Kjeldahl method (N × 6.25) using Kjeltac™ 8400 Auto Sample Systems (Foss Tecator AB, Höganäs, Sweden). Crude lipid content was measured using the Soxhlet method with 500 mL diethyl ether at 50 °C using Soxtoc Avanti 2050 (Foss Tecator AB). Ash content was measured by the combustion method, using a muffle furnace at 550 °C. Amino acid contents of fish meal were determined with an automatic amino acid analyser (L-8900 Hitachi, Tokyo, Japan). Samples were hydrolysed by 6-N hydrochloric acid at 110 °C for 24 h for the determination of amino acids other than sulphur amino acids and tryptophan. For determining sulphur amino acids, samples were first oxidised by formic acid and then hydrolysed by 6-N hydrochloric acid. The tryptophan content was assayed after lithium hydroxide hydrolysis.

**Plasma components and composition of liver analysis**

Plasma glucose, triglyceride (TG), and total cholesterol (T-CHO) content were measured using the glucose oxidase method, the glycerol phosphate oxidase method, and the cholesterol oxidase method, respectively, using test kits (BioSino Bio-technology and Science, Inc., Beijing, China). Hepatic glycogen content was determined as described in Murat and Serfaty (1974). Lipid content of the liver was determined gravimetrically via chloroform-methanol extraction (Wang et al. 1993).

**Enzyme activity analysis**

Liver samples for lipogenic enzymes assays were homogenised in 9 volumes of ice cold 100 mM Tris–HCl buffer containing 0.1 mM EDTA and 0.1% (v/v) Triton X-100 (pH 7.8). Homogenates were centrifuged at 30,000 × g for 30 min at 4 °C. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), Malic enzyme (ME, EC 1.1.1.40) and Fatty acid synthetase (FAS, EC 2.3.1.38) activities were assayed by the methods of Morales et al. (1990), Singer et al. (1990) and Chakrabarty and Leveille (1969), respectively.

Lipoprotein lipase (LPL, EC 3.1.1.34) activity was determined using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China), according to Zheng et al. (2010).

Glucokinase (GK, EC 2.7.1.2) activity was measured using the G6PDH coupling method (Morales et al. 1990). Pyruvate kinase (PK, EC 2.7.1.40) was later
Table 2. Effects of dietary protein and starch levels on the growth performance of Nile tilapia.

| Protein level, % | Starch level, % | IW, g | FBW, g | WG, % | FL, g | FE, % | PER, % | HSI, % | CF |
|-----------------|----------------|-------|--------|-------|-------|-------|------|-------|-----|
| 24              | 0              | 10.29 ± 0.01 <br> 0.000  | 98.86 ± 1.47ab  | 860.60 ± 14.30b  | 194.90 ± 4.50c  | 48.10 ± 0.05a  | 1.87 ± 0.01c  | 2.26 ± 0.02b  | 3.33 ± 0.03c  |
| 20              | 0              | 10.31 ± 0.01 <br> 0.000  | 111.47 ± 0.73b  | 981.20 ± 7.70a  | 187.30 ± 2.90b  | 56.20 ± 0.09b  | 2.22 ± 0.01b  | 2.74 ± 0.12a  | 3.56 ± 0.12b  |
| 40              | 0              | 10.31 ± 0.01 <br> 0.000  | 115.08 ± 0.35  | 1017.10 ± 2.80a  | 179.70 ± 6.20a  | 59.50 ± 0.06bc | 2.41 ± 0.12c  | 2.76 ± 0.13a  | 3.57 ± 0.10b  |
| 36              | 0              | 10.29 ± 0.02 <br> 0.000  | 101.52 ± 0.68a  | 886.40 ± 4.90a  | 161.80 ± 6.10a  | 61.00 ± 0.01  | 1.56 ± 0.02b  | 2.20 ± 0.06a  | 3.81 ± 0.04a  |
| 20              | 0              | 10.28 ± 0.01 <br> 0.000  | 117.26 ± 2.13a  | 1040.10 ± 20.80a  | 169.80 ± 2.90a  | 67.60 ± 0.07c | 1.84 ± 0.04c  | 2.78 ± 0.15a  | 3.94 ± 0.04a  |
| 40              | 0              | 10.29 ± 0.02 <br> 0.000  | 115.87 ± 2.64a  | 1029.50 ± 26.00a  | 143.20 ± 5.00a  | 78.70 ± 0.02d | 2.04 ± 0.06c  | 2.64 ± 0.10a  | 3.88 ± 0.02a  |

ANOVA (p-value)

| Protein level | 0.033  | 0.023  | 0.000  | 0.000  | 0.000  | 0.585  | 0.000  |
| Starch level  | 0.000  | 0.000  | 0.007  | 0.000  | 0.000  | 0.000  | 0.000  |
| Interaction   | 0.307  | 0.327  | 0.546  | 0.478  | 0.798  | 0.744  | 0.161  |

ANOVA: analysis of variance; CF: condition factor; FBW: final body weight; FE: feed efficiency; Fl: feed intake; HSI: hepatosomatic index; IBW: initial body weight; PER: protein efficiency rate; WG: weight gain.

Values with different superscripts in each column indicate significant difference (p < .05).

Data were first analysed using one-way ANOVA to detect differences among all six treatments. A two-way ANOVA was used to test the treatment effects of protein and starch levels on growth performance, whole-body composition, and intermediary metabolic responses. The Kolmogorov–Smirnov test was used to assess the normality of data and the Levene’s test was used to test the homogeneity of variance before using ANOVA. Data expressed as percentage or ratio were subjected to arcsine transformation prior to statistical analysis. A p-value of less than .05 was considered statistically significant.

Results

The results of growth performance are shown in Table 2. Fish receiving 36% protein diets had increased WG, FE, and CF, and decreased Fl and PER compared to fish receiving 24% protein diets regardless of dietary starch level (p < .05). However, at each dietary starch level, similar growth responses were found between fish fed the 36% protein diets and fish fed the 24% protein diets (p > .05). For dietary starch level, an increasing trend of WG, FE, PER, HSI and CF with increasing starch level at 24% protein level was noticed, and the similar response of FE and PER at 36% protein level was found (p < .05). Fl was not affected by starch at 24% protein level, but decreased with increasing starch level at 36% protein level. Feeding non-starch diets led to increased Fl and decreased WG, FE, PER, HSI and CF except for no effect on CF at 36% protein level compared with feeding starch-containing diets (p < .05). Similar responses of WG, HSI, CF and PER were noticed in fish fed diets containing 20% starch and 40% starch, respectively (p > .05). No interaction on growth performance between dietary proteins and starch levels was observed. In addition, the linear regression equation between dietary available energy content (Y) and Fl (X) was fitted (Y = −4.4003X + 228.74, R^2 = .4937, p < .05). There was
a strong negative correlation between dietary available energy content and FI.

Table 3 shows the whole-body composition of tilapia subjected to different dietary protein and starch levels. Feeding 36% protein diets reduced moisture content and increased crude protein content regardless of dietary starch level \((p < .05)\). However, a marginal increase of crude lipid and ash contents was observed when dietary protein level was increased from 24% to 36% \((p > .05)\). A decreasing trend for moisture content at any protein level and ash content at 24% protein level was noted, but an opposite trend for crude protein and lipid contents with increasing dietary starch level was noted.

The plasma and liver components are shown in Table 4. Concentrations of Plasma TG, T-CHO, and glucose, and liver lipid content were significantly higher in fish fed 36% protein diets than those in fish fed 24% protein diets \((p < .05)\). Liver glycogen content was not affected by dietary protein level \((p > .05)\). However, with the exception of liver lipid content at dietary 36% protein, the values for these indices increased \((p < .05)\) with an increase in dietary starch. Significantly higher values for the same indices were observed in fish fed non-starch diets compared with those fish fed starch-containing diets \((p < .05)\). Significant interaction on plasma glucose and liver lipid contents between dietary protein and starch levels was noted, but the effect on other parameters was not observed.

As shown in Table 5, activities of hepatic GK, PK, FAS, G6PDH and ME were higher, but G6Pase and PEPCK activities were lower in fish fed 36% protein diets than those in fish fed 24% protein diets \((p < .05)\), regardless of dietary starch level. Hepatic LPL activity was not affected by dietary protein level. An increasing trend of GK, PK, FAS, LPL, G6PDH and ME activities with increasing dietary starch level, regardless of dietary protein level, was noted. In contrast to the results, however, liver activities of G6Pase and PEPCK displayed the opposite trend. Significant interaction effect on GK, PK, G6PDH and ME was observed between dietary protein and starch levels, but the similar effect did not occur for FAS, LPL, G6Pase and PEPCK.

**Discussion**

All test fish actively forged and survived across the feeding period. It was observed that Nile tilapia fed starch-containing diets had higher WG, FE, PER and HSI compared to fish fed non-starch diets at any dietary protein level, suggesting dietary starch could improve growth and feed utilisation in tilapia. However, no difference of WG was observed between

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**Table 3. Effects of dietary protein and starch levels on whole-body composition of Nile tilapia (%).**

| Protein level, % | Starch level, % | Moisture | Crude protein | Crude lipid | Ash |
|-----------------|----------------|----------|---------------|-------------|-----|
| 24              | 0              | 72.67 ± 0.11<sup>a</sup> | 14.96 ± 0.09<sup>c</sup> | 7.40 ± 0.09<sup>d</sup> | 3.80 ± 0.03<sup>a</sup> |
| 20              | 71.31 ± 0.15<sup>b</sup> | 15.91 ± 0.06<sup>b</sup> | 7.75 ± 0.03<sup>c</sup> | 3.46 ± 0.06<sup>b</sup> |
| 40              | 70.20 ± 0.17<sup>d</sup> | 16.73 ± 0.11a | 8.54 ± 0.07<sup>c</sup> | 3.23 ± 0.05<sup>c</sup> |
| 36              | 0              | 70.66 ± 0.41<sup>c</sup> | 16.46 ± 0.16<sup>a</sup> | 7.60 ± 0.09<sup>d</sup> | 3.75 ± 0.04<sup>a</sup> |
| 20              | 70.55 ± 0.07<sup>c</sup> | 16.71 ± 0.10<sup>a</sup> | 8.08 ± 0.07<sup>c</sup> | 3.49 ± 0.03<sup>b</sup> |
| 40              | 69.62 ± 0.09<sup>d</sup> | 16.66 ± 0.12<sup>a</sup> | 8.38 ± 0.06<sup>a</sup> | 3.51 ± 0.04<sup>b</sup> |

**ANOVA (p-value):**

|             | Protein level | Starch level | Interaction |
|-------------|---------------|--------------|-------------|
| Protein level | 0.000         | 0.000        | 0.055       |
| Starch level | 0.000         | 0.000        | 0.002       |
| Interaction  | 0.008         | 0.000        | 0.912       |

ANOVA: analysis of variance; TG: triglyceride; T-CHO: total cholesterol. Values with different superscripts in each column indicate significant difference \((p < .05)\).

**Table 4. Effects of different dietary protein and starch levels on plasma and liver components of Nile tilapia.**

| Protein level, % | Starch level, % | Plasma, mM | Liver, % | Glycogen, mg/g |
|-----------------|----------------|------------|----------|---------------|
| TG             | T-CHO          | Glucose    | Lipid    | Glycogen      |
| 24              | 0              | 2.64 ± 0.15<sup>c</sup> | 3.23 ± 0.12<sup>c</sup> | 2.81 ± 0.13<sup>c</sup> | 7.44 ± 0.10<sup>c</sup> | 44.11 ± 1.08<sup>b</sup> |
| 20             | 3.61 ± 0.10<sup>c</sup> | 5.02 ± 0.10<sup>c</sup> | 3.16 ± 0.14<sup>c</sup> | 10.80 ± 0.10<sup>c</sup> | 51.65 ± 2.05<sup>b</sup> |
| 40             | 4.14 ± 0.04<sup>a</sup> | 5.30 ± 0.32<sup>a</sup> | 3.17 ± 0.18<sup>a</sup> | 7.60 ± 0.04<sup>a</sup> | 46.45 ± 1.65<sup>a</sup> |
| 36              | 0              | 3.20 ± 0.05<sup>c</sup> | 4.34 ± 0.11<sup>c</sup> | 3.17 ± 0.09<sup>c</sup> | 10.64 ± 0.17<sup>c</sup> | 51.18 ± 1.65<sup>c</sup> |
| 20             | 3.91 ± 0.14<sup>c</sup> | 5.34 ± 0.19<sup>cd</sup> | 3.54 ± 0.17<sup>c</sup> | 10.70 ± 0.27<sup>ca</sup> | 55.45 ± 5.7<sup>a</sup> |
| 40             | 4.77 ± 0.08<sup>b</sup> | 5.55 ± 0.13<sup>b</sup> | 3.54 ± 0.17<sup>a</sup> | 10.70 ± 0.27<sup>ca</sup> | 55.45 ± 2.17<sup>a</sup> |

**ANOVA (p-value):**

|             | Protein level | Starch level | Interaction |
|-------------|---------------|--------------|-------------|
| Protein level | 0.000         | 0.001        | 0.000       |
| Starch level | 0.000         | 0.000        | 0.000       |
| Interaction  | 0.285         | 0.102        | 0.000       |

ANOVA: analysis of variance; TG: triglyceride; T-CHO: total cholesterol. Values with different superscripts in each column indicate significant difference \((p < .05)\).
starch-containing treatments for each protein level, implying that dietary 20% starch is enough for normal growth, which is consistent with previous studies (Zhou et al. 2015). In this study, the lower protein (24%) diets led to marginal decreased WG and FE but increased PER compared to the higher protein (36%) diets regardless of dietary starch level, which is in agreement with previous results (Shiau & Peng 1993). The little growth difference may result from high-quality protein source that was provided only by fish meal. This is because fish meal used in our study was produced through the steam process and thus has a high biological value and a well-balanced amino acid profile (NRC 2011). PER increased with increasing dietary starch level at each dietary protein level, and the fish fed the 24% protein diets containing 20% or 40% starch had similar WG comparable to fish fed the 36% protein diets containing 0 or 20% starch in the present study. As indicated earlier, an obvious protein-sparing effect of starch was also rendered on growth performance in tilapia (Shiau & Peng 1993), and other fish species such as turbot (Scophthalmus maximus; Zeng et al. 2015). Fish have a certain ability to adjust the amount of feed intake according to feed energy level (NRC 2011). In the present study, increasing protein and/or starch level resulted in decreased FI as a result of increase of dietary available energy, which showed FI, to a certain extent, was regulated not only by dietary protein and starch but by the dietary energy level in fish. Our results showed that the compensation of non-starch diets by a little increase in FI in tilapia was not sufficient to reach the same amount of energy intake as with starch-containing diets. These findings indicated inferior growth performance in fish fed the non-starch diets vs. fish fed the starch-containing diets was associated with insufficient energy supply due to dietary starch deficiency, showing the importance of dietary starch in the nutrition of tilapia.

The highest whole-body moisture and ash contents and lowest whole-body protein and lipid contents were observed in fish fed non-starch diets among dietary starch treatments regardless of dietary protein level, indicating a poor protein and lipid retention. The opposite result is observed in fish fed the diets at the highest starch level. Considering these results, it appears that a decrease in whole-body moisture content is at the expense of an increase in whole-body crude lipid content with regard to dietary starch level (Chen et al. 2012). Besides starch, high level of other forms carbohydrates could also stimulate whole-body lipid retention in Nile tilapia (Gaye-Siessegger et al. 2006) and grass carp (Ctenopharyngodon idella; Chen et al. 2012). Although FI was negatively correlated with dietary energy level, the increasing trend of whole-body lipid content was positively associated with the increase in energy intake at each dietary protein level in the present study. A positive relationship between whole-body protein retention and dietary carbohydrate was observed in the current study with 24% protein diets, suggesting that carbohydrates can be effectively promoted tissue protein deposition in a larger extent (Chen et al. 2012; Vásquez-Torres & Arias-Castellanos 2013). But deposition of whole-body protein was not affected by dietary starch level when fish were fed a diet with 36% protein, indicating wastage of excessive dietary protein with greater protein catabolism.

It is generally accepted that low utilisation of carbohydrate results from a dysfunction in the nutritional regulation of carbohydrate metabolic pathways in the liver and absence/low activity of several key enzymes (such as GK, PK, PEPCK, G6Pase) in the carbohydrate metabolism of fish (Hemre et al. 2002). In our study, liver GK activity improved with increasing dietary starch levels, which was similar to previous studies (Leung & Woo 2012). In addition, plasma glucose level was higher in fish fed the carbohydrate-rich diets, indicating more energy intake. This further verified our above observations that whole-body lipid content was increased with increasing dietary energy intake due to the increase in starch intake. The findings show a close agreement with previous results (Shiau & Peng 1993).
relationship between plasma glucose level and whole-body lipid deposition with regard to dietary carbohydrate levels. As a consequence, glucose uptake is stimulated in the liver, an enhancement in hepatic (PK and GK) enzyme activities of glycolysis pathway (Caseras et al. 2002), thus contributing an increase in liver glycogen content to maintain glucose homeostasis (Azaza et al. 2015). This is further verified by the results of previous studies (Coutinho et al. 2015). In terms of effect of dietary protein level, higher GK and PK values were observed in fish fed the high-protein diets versus the low-protein diets in this study. By contrast, higher GK and PK values were found in low-protein diet fed in blackspot seabream (Pagellus bogaraveo; Figueiredo-Silva et al. 2009). These carnivorous fish may acquire energy to meet demands by means of increased glycolysis when fed low-protein diets. Although there was no modification of PEPCK, and G6Pase gene expression and/or activities with regard to dietary carbohydrates (Caseras et al. 2002; Coutinho et al. 2015) and/or protein (Kirchner et al. 2005), lower G6Pase and PEPCK activities in fish fed high-protein and/or high-starch diets compared to lower level were observed in the current study, indicating a reduction in gluconeogenesis in Nile tilapia, which is in agreement with previous results (Guerreiro et al. 2014; Enes et al. 2015). Both the increase in glycolysis and reduction in gluconeogenesis are the way to keep glucose homeostasis by decreasing the plasma glucose level (Enes et al. 2015). This phenomenon was also observed as protein and/or carbohydrate intake increased, thus enhanced energy intake in our current study.

Higher dietary carbohydrate levels have shown to stimulate activities of liver G6PDH and ME in Nile tilapia (Azaza et al. 2015) and in this study. A positive relationship between liver G6PDH and/or ME activity and liver lipid deposition was noted in zebra seabream (Diplodus cervinus; Coutinho et al. 2015) with regard to dietary carbohydrate levels, which was further supported by the results of the current study. Additionally, the data from the current study showed a positive relationship between plasma glucose, TG, and T-CHO levels, liver FAS, G6PDH, ME or/and LPL activities and dietary starch level, and such is the relationship between the same indices and dietary protein level. These responses also responded to higher whole-body lipid deposit, liver lipid, and glycogen deposit and HSI when the fish were fed the diets with higher starch or/and protein levels, which was in line with previous observations (Fernández et al. 2007; Zhou et al. 2013; Zeng et al. 2015), i.e. ingested carbohydrates in a small amount (<20%) could be converted into glycogen in the liver and in the muscle, and even a small part of which is converted into liver lipids. The data from the current study demonstrate that the fate of excess glucose from excessive dietary carbohydrate supply may be either toward synthesis of glycogen or toward lipogenesis to store energy. The high levels of plasma glucose, TG, and T-CHO, and high activities of liver FAS, G6PDH, ME and LPL are a reflection of enhanced lipid metabolism in fish fed higher protein and/or starch diets, indicating a consequence of excessive energy intake. Therefore, excessive dietary energy could be stored in the liver and/or other tissues as lipid or glycogen in tilapia fed high dietary protein and/or starch diets in this study.

Conclusions

In conclusion, absence of starch in diets may result in relatively poor nutritional status of Nile tilapia due to the insufficient energy supply, and appropriate addition of starch in diets could obtain better growth. However, although dietary starch higher than 20% did not further promote growth, it led to whole-body lipid and liver glycogen deposit. The protein-sparing effect of starch on Nile tilapia growth exists and can be enhanced when dietary protein is low. Insufficient dietary protein (24%) allowance does not significantly affect the growth of Nile tilapia when dietary protein is provided only by fish meal as dietary protein source with at least 20% starch supplementation.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant number 31372546] and the Science and Technology Major Project of Fujian Province [grant number 2016NZ0001-3].

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 31372546] and the Science and Technology Major Project of Fujian Province [grant number 2016NZ0001-3].

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