Effect of dietary lipid levels on body compositions, digestive ability and antioxidant parameters of common carp

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Abstract. This study was designed to evaluate the effect of dietary lipid level on body composition, and digestive ability of common carp with initial average weight (36.12 ± 1.18)g. Five experimental diets with increasing lipid levels of 2.1%, 4.0%, 5.8%, 7.6%, 9.4% were fed to triplicate groups of fish for 9 weeks. The results showed that lipid content of whole body and muscle increased in parallel with the increase of dietary lipid levels. Protein content of muscle decreased with the increase of dietary lipid levels, and the lowest muscle protein content was observed in fish fed 9.4% lipid diet. Lipase activity was significantly affected by dietary lipid levels in hepatopancreas and intestine (P < 0.05). Lipase activity in fish fed at 5.8% lipid level group was significantly higher than others in hepatopancreas (P < 0.05). There were no significant differences in amylase and protease activities (P > 0.05). The results suggested that the most excellent digestive ability and antioxidant parameters were obtained at 7.6% lipid level group.

1. Introduction

Over the last few decades, finding best practices on how to decrease fish meal consumption and improve the protein utilization by fish has become an environmental and economical goal for the sustainable development of aquaculture feed industry [1,2]. Therefore, many studies have been designed to determine and boost the protein-sparing feasibility of lipid and carbohydrates in fish diets [3, 16, 22]. Lipid plays an important role on maintaining the fish growth, development and normal physiological metabolism. Accordingly, whether dietary lipid level is appropriate or not have an effect on the growth of fish. Related research shows that in many fish there are a positive correlation between dietary lipid levels and lipid of fish body and muscle [5,6]. Also dietary lipid levels will have an impact on moisture, ash, protein content of fish body and muscle, and especially effects on protein content also reflect the saving function of lipid to protein from the side [13].

In addition, some studies have shown that as a substituted source, a recruitment of lipids rather than carbohydrates is generally a much more productive way to save the dietary protein because fish acquire a “glucose intolerant” predisposition, leading to poorer ability to utilize carbohydrate [14]. However, it is worth noting that studies of most fish species concentrated on the impacts of dietary
lipid levels on growth performance and body composition [4], the impacts on digestive ability has received limited attention.

Common carp (Cyprinus carpio), an omnivorous fish, are native to Asia. The present research on lipid nutrition of this species mainly focuses on the requirement of the total quantity and lipidty acids, whereas few studies have been conducted on digestive ability and antioxidant parameters to dietary lipid levels [18, 24]. Bearing this in mind, the present study was conducted to evaluate the effects of dietary lipid levels on body compositions, digestive ability of common carp.

2. Materials and methods

2.1. Experimental diet

Five experimental diets were formulated with increasing lipid levels of 2.1%, 4.0%, 5.8%, 7.6%, 9.4% (Table 1). All ingredients were provided by Tianjin Tianxiang Aquatic Co. Ltd., and were pulverized by 60 mesh sieve, thoroughly mixed, and made into 2 mm pellets using a pellet machine (MUZLMV4, Jiangsu Muyang Group Co., Ltd., Yangzhou, China). The experimental diets were stored at -20℃ until used.

| Ingredients          | Diet lipid levels, % |
|----------------------|----------------------|
|                      | 2.1  | 4.2  | 5.8  | 7.7  | 9.8  |
| Fish meal            | 6    | 4.5  | 3    | 1.5  | 0    |
| Soybean meal         | 22   | 22   | 22   | 22   | 22   |
| Peanut meal          | 12   | 12   | 12   | 12   | 12   |
| Cotton meal          | 10   | 10   | 10   | 10   | 10   |
| Rapeseed meal        | 12   | 12   | 12   | 12   | 12   |
| Cellulose            | 2    | 1.5  | 1    | 0.5  | 0    |
| DDGS                 | 3    | 3    | 3    | 3    | 3    |
| Soybean oil          | 0    | 2    | 4    | 6    | 8    |
| Premix¹              | 4    | 4    | 4    | 4    | 4    |
| Wheat flour          | 9    | 9    | 9    | 9    | 9    |
| Wheat middlings      | 10   | 10   | 10   | 10   | 10   |
| Wheat bran           | 10   | 10   | 10   | 10   | 10   |
| Total                | 100  | 100  | 100  | 100  | 100  |
| Proximate composition¹², % of dry matter basis |
| Dry matter           | 89.8 | 89.8 | 89.9 | 89.7 | 89.7 |
| Crude protein²       | 32.2 | 31.1 | 30.2 | 29.2 | 28.3 |
| Ash                  | 7.6  | 7.5  | 7.3  | 7.0  | 6.8  |
| Crude lipid          | 2.1  | 4.2  | 5.8  | 7.7  | 9.4  |
| Gross energy³, MJ/kg | 13.7 | 14.2 | 14.6 | 15.1 | 15.65 |

¹ Premix contains per kg diet: vitamin A 1,500 IU, vitamin B₁ 4.5 mg, vitamin B₂ 5 mg, vitamin B₆ 3.75 mg, vitamin B₁₂ 5 mg, vitamin D₃ 500 IU, vitamin E 25 mg, vitamin K₁ 2.5 mg, biotin 0.03 mg, folic acid 1 mg, D-pantothenic acid 5 mg, nicotinic acid 25 mg, inositol 20 mg, vitamin C 30 mg, antioxidant 1 mg, Cu (as copper sulfate) 2.25 mg, Fe (as ferrous sulfate) 22.5 mg, Mn (as manganese sulfate) 0.5 mg, Zn (as zinc sulfate) 12.5 mg, I (as potassium iodide) 0.1 mg, Se (as sodium selenite) 0.005 mg, Co (as cobalt chloride) 0.075 mg.

² By analysis.

³ By calculation. Calculated as protein: 24 kJ/g; fat: 38 kJ/g; and starch: 17 kJ/g.
2.2. Experimental fish and procedure
Common carp were obtained from Tianjin Huanxin Aquatic Breeding Farm. Firstly, fish were acclimated to the experimental conditions and fed a commercial formulated feed (32% protein) for 2 weeks in the 3 m×3 m×1.5 m cages. Before the beginning of feeding trial, fish were fasted for 24 h. 60 fish individuals each cage and 3 cages each diet. During the 9-week feeding trial, all groups were fed daily at on a 4% body weight ratio twice daily (9:00 and 15:30). Water quality parameters during the experimental period were pH 7.8 ± 0.2, temperature 27.9℃ to 30.1℃, dissolved oxygen 6.0 to 8.0 mg/L and total ammonia nitrogen<0.2 mg/L.

2.3. Sample collection
At the end of the trial, fish were fasted for 24 h before sampling. For a whole-body composition analysis, 6 fish from each cage were randomly sampled and analysed. Ten fish per cage were firstly collected for blood collection from the caudal vein. Blood samples were immediately centrifuged at 3,170 g for 15 min at 4℃, and the obtained serum was stored at -80℃ until the biochemical criterion determinations were made. The bloodless fish were immediately dissected by operating at ice plate for dorsal muscles hepatopancreas, foregut, midgut and hindgut sampling.

2.4. Analytical methods
2.4.1. Proximate composition. The contents of moisture, crude protein, crude lipid, and crude ash (wet weight) were determined in prepared samples of experimental diets, whole body and dorsal muscles which were frozen at -20℃. Moisture, crude protein, crude lipid, and crude ash were determined following standard methods.

2.4.2. Analysis of digestive enzyme activity in foregut, midgut and hindgut. For the digestive enzymes, tissue samples were homogenized in 4 volumes of ice-cold physiological saline (0.85% w/v NaCl). Homogenates were centrifuged at 3,500 g for 15 min at 4℃, and the resulting supernatants were aliquoted and stored at -80 ℃ until subsequent analysis.

Protease activity was assayed by Folin-reagent method (Khantaphant and Benjakul 2008). The activities of intestine and hepatopancreas lipase and amylase were determined using diagnostic reagent kits provided by Jiancheng Bioengineering Institute (Nanjing, China). Enzyme activities were expressed as U/g tissue.

2.5. Statistical analysis
All data were expressed as mean values ± standard error (SE) and subjected to one-way analysis of variance (ANOVA). Percentage data were arcsine transformed before analysis of variance. When there were significant differences, Duncan’s multiple range tests were conducted among group means. The significant level was set as $P < 0.05$. All statistical analyses were performed using the SPSS 17.0.

3. Results
3.1. Effects of dietary lipid levels on nutrient composition of common carp
As can be seen from Table 2, whole body crude protein and crude lipid concentrations were enhanced with an increase in dietary lipid levels in experimental group. Furthermore, fish fed the diets containing 4.2 to 9.4% lipid levels showed higher crude protein contents in comparison to fish was fed the diets containing 2.1% lipid level ($P < 0.05$). Liver lipid concentrations reached 8.14 ± 0.17% at 7.7% lipid level which was significantly higher than that at other lipid levels ($P < 0.05$). In addition, moisture ranged from 74.19% to 75.44% and ash contents ranged from 2.72% to 2.91% without marked differences between the different dietary lipid levels ($P > 0.05$).

The contents of moisture, crude ash and crude lipid in the muscles increased with increasing dietary lipid, but the contents of moisture and crude ash were similar in the muscles ($P > 0.05$). In turn, significantly higher contents of crude lipid and lower contents of crude protein were noted in the muscles of the fish fed the diets containing 7.7% lipid level in comparison with the other dietary
treatments. With increasing dietary lipid levels, the contents of crude protein tended to decrease, and there were significant differences among all the experimental groups ($P < 0.05$). Table 2 is the same.

### 3.2. Effects of dietary lipid levels on digestive enzyme activity of common carp

Protease activities of common carp fed diets containing increasing levels of lipid are presented in Table 3. The activities of protease were observed to decrease compared to the experimental diets in foregut, midgut and hindgut and hepatopancreas. In the whole intestine, protease activities of midgut were higher in comparison to protease activities of foregut and hindgut, while the statistical analysis computed for protease activities of foregut, midgut and hindgut under different experimental treatments did not detect any significant differences ($P > 0.05$). However, an apparent effect was present on protease activity of hepatopancreas, which decreased significantly at 7.7% and 9.4% lipid level groups ($P < 0.05$).

Lipase activity of common carp fed diets containing graded levels of lipid are summarized in Table 4. The activities of lipase in foregut, midgut and hindgut and hepatopancreas represented a trend to decline after the first rise with increasing inclusion of lipid. In the whole intestine, lipase activities of midgut were higher compared to lipase activities of foregut and hindgut. Besides, the lipase activities of foregut, midgut and hindgut of common carp at 7.7% lipid level group were significantly higher than those of the 2.1%, 4.2% and 9.4% lipid level groups ($P < 0.05$). Moreover, the fish at 5.8% lipid level group showed a significant higher lipase activity in their body compared with the other groups ($P < 0.05$).

### Table 2 Effects of dietary lipid levels on nutrient compositions of the whole body and muscle(%)

| Proximate composition | Lipid level, % | 2.1  | 4.2  | 5.8  | 7.7  | 9.8  |
|-----------------------|----------------|------|------|------|------|------|
| Whole body            |                |      |      |      |      |      |
| Crude protein         |                | 15.13±0.10$^b$ | 16.33±0.03$^a$ | 16.64±0.27$^a$ | 16.23±0.44$^a$ | 16.65±0.68$^a$ |
| Crude lipid           |                | 6.15±0.03$^d$ | 6.66±0.20$^c$ | 7.67±0.21$^b$ | 8.14±0.17$^a$ | 7.67±0.19$^b$ |
| Crude ash             |                | 2.74±0.12 | 2.91±0.02 | 2.89±0.06 | 2.72±0.11 | 2.72±0.05 |
| Moisture              |                | 74.47±1.80 | 75.44±1.22 | 74.19±2.88 | 74.40±1.67 | 74.36±1.18 |
| Muscle                |                |      |      |      |      |      |
| Crude protein         |                | 20.17±0.03$^b$ | 20.40±0.07$^a$ | 19.95±0.04$^c$ | 19.18±0.09$^d$ | 18.76±0.08$^c$ |
| Crude lipid           |                | 2.54±0.17$^b$ | 2.39±0.07$^b$ | 2.19±0.09$^c$ | 2.61±0.69$^b$ | 2.83±0.23$^a$ |
| Crude ash             |                | 1.17±0.06 | 1.34±0.04 | 1.32±0.13 | 1.28±0.27 | 1.15±0.08 |
| Moisture              |                | 79.05±0.01 | 78.02±0.03 | 79.12±0.02 | 79.97±0.04 | 80.01±0.02 |

1 Values are means ± SE. $^a$–$^e$ Values in the same row with different superscripts are significantly different ($P < 0.05$).

Amylase activities of common carp are summarized in Table 5. The activities of amylase in foregut, midgut and hindgut and hepatopancreas represented a downward trend after the first rise with increasing inclusion of lipid, while increasing dietary lipid levels did not cause significant difference in amylase activities under different experimental treatments ($P > 0.05$).

### Table 3 Effects of dietary lipid levels on protease activity of common carp (U/mg protein)

| Tissues            | Lipid level, % | 2.1  | 4.2  | 5.8  | 7.7  | 9.8  |
|--------------------|----------------|------|------|------|------|------|
| Hepatopancreas     |                | 82.70±1.94$^a$ | 80.96±1.87$^a$ | 80.23±2.00$^b$ | 73.97±4.11$^b$ | 74.09±3.42$^a$ |
| Foregut            |                | 66.34±5.44 | 65.31±5.51 | 66.84±2.86 | 63.54±4.49 | 63.56±7.00 |
| Midgut             |                | 106.16±8.71 | 103.89±7.11 | 100.79±5.50 | 99.11±5.15 | 95.98±6.92 |
| Hindgut            |                | 71.15±4.93 | 70.15±5.86 | 70.05±6.09 | 65.89±9.10 | 65.75±7.14 |

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Table 4 Effects of dietary lipid levels on lipase activities of common carp (U/mg protein)

| Tissues       | Lipid level, % |
|---------------|----------------|
|               | 2.1            | 4.2            | 5.8            | 7.7            | 9.8            |
| Hepatopancreas| 103.84 ± 5.64^b| 108.24 ± 6.38^b| 142.94 ± 10.54^a| 58.93 ± 6.81^c| 48.45 ± 5.12^c|
| Foregut       | 114.15 ± 8.21^b| 127.29 ± 9.58^b| 166.06 ± 12.95^b| 149.98 ± 13.71| 111.68 ± 9.46^b|
| Midgut        | 34.75 ± 3.27^a| 34.58 ± 3.09^c| 68.87 ± 7.77^a| 73.24 ± 3.08^a| 52.11 ± 5.32^b|
| Hindgut       | 116.26 ± 4.82^b| 124.77 ± 4.81^b| 132.65 ± 1.58^a| 139.91 ± 5.49^a| 69.62 ± 1.54^c|

Table 5 Effects of dietary lipid levels on amylase activities of common carp (U/mg protein)

| Tissues       | Lipid level (%) |
|---------------|-----------------|
|               | 2.1            | 4.2            | 5.8            | 7.7            | 9.8            |
| Hepatopancreas| 12.24 ± 2.96    | 13.37 ± 3.97   | 11.84 ± 4.81   | 11.33 ± 3.35   | 10.43 ± 1.98   |
| Foregut       | 14.43 ± 4.01    | 17.15 ± 4.36   | 20.54 ± 4.76   | 17.76 ± 4.33   | 12.12 ± 3.10   |
| Midgut        | 8.13 ± 1.77     | 9.10 ± 2.28    | 10.43 ± 3.10   | 8.62 ± 2.57    | 10.26 ± 3.76   |
| Hindgut       | 5.12 ± 1.13     | 5.86 ± 1.24    | 6.59 ± 1.35    | 6.60 ± 1.17    | 4.93 ± 1.09    |

4. Discussion and conclusions

Currently, many scholars have carried on the related and inter-depth research and reports on the relationship between dietary lipid levels and body composition. Generally speaking, the relationship between the lipid content of the whole body of fish is positively correlated, and the abdominal cavity, liver, pancreas and muscle tissue is the part of the excess lipid deposition. Thus, this will cause a lot of adverse effects, such as decline of meat quality, decline of storage stability, and then affect its economic value [19]. In this experiment, fish fed the diets containing 4.2% to 9.4% lipid levels showed higher crude lipid contents in comparison to fish fed the diets containing 2.1% lipid level (P < 0.05), which indicated that the lipid deposition of fish body will be enhanced with an increase in dietary lipid levels. The lipid content of the muscle of some fish does not vary with changes of dietary lipid levels, such as the European sea bass (*Dicentrarchus labrax*) [11]. However, in this trial, lipid content of muscle increased significantly with increasing levels of dietary lipid, indicating that part of the lipid could deposit in muscle tissue, and the above results were similar to the results of the study of Zhu et al [27] on *Siganus guttatus* and the study of Peng et al [10] on *Lutjanus erythopterus*. Relative to the carnivorous fish, lipid deposition ability of the omnivorous fish is stronger than the former, and therefore after meeting their own needs, the excess lipid is not as the energy source to consumption, but was accumulated in the guts, which is why the hepatosomatic indexes (HSI) of experimental common carp fed with five experimental diets increased gradually with increasing dietary lipid levels [23]. In addition, from the perspective of watching crude protein, crude protein content of the whole body of fish with increasing dietary lipid level increased, while decreased in the muscle, and affect significantly (P < 0.05), indicating that to increase dietary lipid levels within a certain range can reduce the consumption of the protein as energy source so that more protein are used for the growth of fish and body protein synthesis. The above result has also been reported in the study on yellow croaker *Larimichthys crocea*[17]. Wang et al.[20] found that dietary lipid levels less than 10% had no significant effect on the crude protein content of the muscles of *Carassius auratus gibelio*, not consistent with with the results of this study, which resulted from the lipid source and the lipid level of dietary and fish species. The related mechanisms will be examined in-depth in future studies. This study also found that no significant differences were noted in the quantity of moisture and crude ash in the muscles of the experimental fish of which the dietary lipid levels ranged from the 2.1% to 9.4%, consistent with previous reports for *Squaliobarbus curriculus*[26].

Digestion is the first limiting factor, which have an effect on utilizing diets for growth. Therefore, fish digestive enzyme is known as an important part of digestive physiology research. In the present
study, decline in protease activities of foregut, midgut and hindgut and hepatopancreas were found with increasing inclusion of lipid, whereas lipase activities of these represented an upward tendency after the first decline. Two explanations can be advanced to reveal this result. For one thing, this suggest that appropriate lipid can provide the well-stocked supply of raw materials for the synthesis of essential lipid acids, and satisfy the individuals energy requirements so that the amount of dietary protein used for the energy consumption can be able to minimized by a supplementation of lipids. This was also certificated by no significant different among the different experimental treatments in the present study. Similar findings were also found in Chinese sucker (Myxocyprinus asiansicus) [21] and Siganus guttatus [25]. For another thing, the present study may suggest within the optimum range (2.17% to 7.67%) of dietary lipid in the diets of common carp, A positive correlation between lipase activity intestine and hepatopancreas of and dietary lipid levels was observed, revealing that improvements in lipase activity may be attributable to the higher availability of dietary lipid as substrate in an optimum range, in line with the results obtained in Megalobrama amblycephala [9]. Different results were observed in yellow catfish (Pelteobagrus vachelli) [15], and jade perch (Scortum bacoo) [12]. That might be attributed to the difference of feeding habits, growth phase, dietary carbohydrate sources and so on. However, further studies should be needed to reveal this.

The most excellent protein content of the whole body and muscle, digestive ability and antioxidant parameters was obtained at 7.6% lipid level group.

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