Effects of dietary protein levels on the long-term growth response and fitting growth models of gibel carp (Carassius auratus gibelio)

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ABSTRACT

A 41-wk growth trial was conducted to evaluate the effects of dietary protein levels on the long-term growth response and fitting growth models of gibel carp (Carassius auratus gibelio) with an initial body weight of 1.85 ± 0.17 g. The dietary protein levels were designed at 320 (P32), 360 (P36), 400 (P40), and 440 g/kg (P44), respectively. The growth curves of the gibel carp for each group were fitted and analyzed with four nonlinear regression models (Gompertz, logistic, von Bertalanffy and Richards). The final body weights (mean ± SD) of the fish were 226 ± 6, 231 ± 7, 242 ± 2, and 236 ± 2 g for P32, P36, P40, and P44, respectively. Feed conversion ratio of P40 and P44 groups was significantly lower than that of P32 and P36 groups (P < 0.05). Productive protein value of P44 group was significantly lower than that of P32 and P36 groups, but not different from that of P40 group (P > 0.05). The growth response of the gibel carp for each group was the best fitted by Richards model with the lowest Chi², residual sum of squares and residual variance, then Gompertz and von Bertalanffy growth models, but the logistic model did not fit the data well justified by Chi² values. The optimal protein level (400 g/kg) prolonged the stage of fast growth and predicted the highest asymptotic weight, which was close to the harvest size in practice.

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1. Introduction

A statistical method is important for estimating nutrient requirement. Shearer (2000) reported that ANOVA and the broken-line model were the most frequently used to determine dietary nutrient requirements of aquaculture species. However, both of ANOVA and broken-line model are inadequate for describing the dose–response of a population (Morris, 1999; Shearer, 2000).

Growth curves describe the regular changes in live weight or in a particular body part of an animal with increasing age (Ricker, 1979; Pauly, 1981; He and Stewart, 2002). In animals, growth curves are generally S-shaped and based on long-term growth datasets (De Graaf and Prein, 2005; Ersoy, 2006; Yang et al., 2006). Gompertz, logistic, Richards and von Bertalanffy models are often used to fit growth curves of fish, especially for the time-growth response estimation, which much better than ANOVA or broken-line models (Jiang and Qin, 1996; Gamito, 1997, 1998; He and Stewart, 2002; Hemandex-Llamas and Rakowsky, 2004; Ersoy, 2006; Russo, 2009). Studies on long-term growth curves of animals are of major importance for dynamically understanding the process of growth and responses on dietary nutrient density as well. The resulting information can be easily used to guide feeding and management programs (De Graaf and Prein, 2005; Yang, 2006; Russo, 2009).

The gibel carp (Carassius auratus gibelio) is an important omnivorous species cultured and for wild tagging in China, and widely spreads in some countries of Asia and Europe. It is a
2. Materials and methods

2.1. Feed ingredients and diet formulation

Fish meal and soybean meal were used as the primary protein sources. Fish oil and soybean oil were used as the lipid sources. Wheat flour and wheat middling were used as sources of carbohydrates. Four iso-energetic (approximately 17.4 MJ/kg) practical diets were formulated to contain 320, 360, 400 and 440 g/kg crude protein (CP) with similar essential amino acids (EAA) proportions (Table 1).

### Table 1

Ingredients and nutrient composition of the experimental diets, g/kg.

| Item                  | P32   | P36   | P40   | P44   |
|-----------------------|-------|-------|-------|-------|
| Moisture              | 88.2  | 89.6  | 88.1  | 86.1  |
| CP                    | 320   | 358   | 388   | 431   |
| Crude lipid           | 54.4  | 55.2  | 53.1  | 54.1  |
| Gross energy, MJ/kg   | 17.3  | 17.5  | 17.5  | 17.9  |
| Amino acids proportion|       |       |       |       |
| Lys/CP                | 0.07  | 0.07  | 0.07  | 0.07  |
| Met/CP                | 0.02  | 0.02  | 0.02  | 0.02  |
| Met/Lys               | 2.89  | 2.76  | 2.82  | 2.88  |

Groups of P32, P36, P40 and P44 denoted dietary protein levels 32, 36, 40 and 44 g/kg, respectively.

2.2. Fish and experimental conditions

The gibel carp (female) were obtained from the Huanxin Fish Farm (Tianjin, China) and acclimated to the recirculating system for 2 wk by feeding a commercial diet containing 39% CP and 17.4 MJ/kg gross energy (Enhalor Group, Beijing, China) before the trials. Gibel carp (initial body weight: 1.85 ± 0.17 g) were randomly distributed into 20 tanks with a conical bottom (diameter: 80 cm; volume: 0.3 m³). Five replicates were randomly assigned to each diet group, and 50 fish were batch weighed and stocked in each tank after 24 h starvation. During the 41-wk feeding period, the fish were weighed every two or three weeks, and the fish were distributed to larger tanks (0.8 m³) for keeping the rearing density at appropriate level at the end of the 18th week to maintain normal growth performance. The fish were fed the experimental diets to apparent satiation four times daily at 0800, 1100, 1400 and 1700, the same feeding protocol as farming practice. At each feeding, the water flow was stopped, and an excess quantity of weighted diet was provided. One hour later, the uneaten diet was removed, dried to constant weight at 70 °C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying and reweighing. Feed intake of fish in each tank was calculated as the difference between the amount fed and the amount of uneaten diet recovered, corrected for leaching loss.

During the feeding period, experimental fish were compiled with Laboratory Animal Welfare Guidelines of China (Decree No. 2

\*1 Fish meal and fish oil were produced in Peru and supplied by the International Fish Meal and Fish Oil Organization (IFFO, Hertfordshire, UK); soybean meal, full-fat extruded soybean, soybean oil and lecithin were supplied by Yihai Kerry Investment Company Limited; Shandong, China; wheat flour and wheat middling were supplied by Guchan Group, Beijing, China; Other ingredients and vitamin and mineral premix (mg/kg diet) were supplied by Shanghai Enhalor Biotech Ltd. Co. Beijing, China.

\*2 Vitamin premix supplied the diet with (mg/kg diet) the following: retinyl acetate 28; cholecalciferol 14; vitamin E (50%) 300; vitamin K 4; thiamin HCl; riboflavin 8; pyridoxine hydrochloride 14; vitamin B12 (1%) 0.1; L-ascorbyl-2-monophosphate-Na 600; calcium pantothenate 100; amine nicotinic acid 80; folic acid 2; inositol 200; choline chloride (50%) 3000; wheat middling 1648; Mineral premix consisted of (mg/kg diet) the following: FeSO4·7H2O 750; ZnSO4·7H2O 350; CuSO4·5H2O 25; MnSO4·4H2O 200; KI 5; CoCl2·6H2O 2.5; Na2SeO3 5; MgSO4 1000; zoonol 1.663.

Growth of gibel carp during the long-term feeding periods occurring in the aquaculture of this species.

Protein is the most important component of fish feeds. Increasing the protein level in the feeds can improve fish production, but excessive dietary protein will be metabolized as an energy source and produce more nitrogen discharge (Tibbetts et al., 2000). This process may be detrimental to fish growth. Therefore, the knowledge of the protein requirement of fish is essential for the formulation of well-balanced and low-cost diets. Most data of nutrients requirement of gibel carp have been published (Luo et al., 2004; Xue et al., 2012). This process may be detrimental to fish growth. Therefore, the knowledge of the protein requirement of fish is essential for the formulation of well-balanced and low-cost diets. Most data of nutrients requirement of fish are based on a short-term (such as an 8–12 wk growth trial) study, and in which, most of them are started with small size fish (Qian, 2001; Giri et al., 2003; Li, 2008). Qian (2001) reported that the protein requirement of juvenile gibel carp with initial body weight (IBW) of 4.78 g are 35, 38.4 and 48.6% analyzed by ANOVA, broken-line or quadratic models, respectively by an 8-wk growth trial. Besides, Zhou et al. (2005) established an energetic model to estimate the feed requirement of gibel carp with a diet of 39% protein level by a 31-d feeding trial. However, the criticisms of the above studies are large variety of the results and the problem of not enough old fish to accurately characterize the asymptote in growth models by the short-term growth trial (Francis, 1988). Jiang et al. (1996) determined the growth curve with von Bertalanffy model for wild-caught crucian carp in Dali lake of China. Besides, growth curves had been established for red grouper (Epinephelus morio) (Jones, 2000; Björnsson, 1995) and gilthead sea bream (Sparus aurata) (Gamito, 1998). The modeling growth curves determined under natural conditions might vary with climate, temperature and other environmental changes, and such variation might reduce the precision with which the growth curves can be assessed. Moreover, one of the most important factors affecting fish growth under intensive aquaculture is the quality of the feed. The objective of the present study was, therefore, to re-evaluating the protein requirement of gibel carp by fitting the growth curves with four types of models (Gompertz, logistic, von Bertalanffy and Richards) in a long-term growth trial.
of Ministry of Science and Technology, issued in 1988). The water temperature was maintained at 22–24 °C, the pH was 7.5–8.0, ammonia nitrogen was lower than 0.1 mg/L, nitrite was lower than 0.1 mg/L, and continuous aeration ensured that dissolved oxygen remained at levels >6.0 mg/L.

2.3. Adopted growth models

Four nonlinear growth models, the logistic, Gompertz, von Bertalanffy and Richards were applied to analyze the data (Table 2). The absolute growth rate (AGR), body weight at inflection point (BWI) and weeks of inflection (WI) were calculated according to the first derivative and second derivative of the model equations.

2.4. Statistical analyses

These data were expressed as means of 5 replicates at each time point. Bartlett’s Box F test was used for homogeneity test, and there were no differences among variances. Significant differences (P < 0.05) of each variable were firstly detected the body weights of various sampling time point in one-way ANOVA test, and then Duncan’s multiple range test was used to rank the group by STATISTICA 8.0 software. A Bonferroni adjustment for the comparison of means was performed to account for multiple testing. Significance was defined at P < 0.05.

3. Results

The growth performances of gibel carp fed the experiment diets over a period of 41 wk are reported in Table 3. Survival for each group was 100%. Dietary protein levels significantly affected growth and feeding behavior. Final weight and specific growth rate (SGR) were higher in fish fed diet P40 than those in fish fed diet P32. Feed intake (FI), feed conversion ratio (FCR) and productive protein value (PPV) were significantly decreased with higher dietary protein levels. FCR of P40 and P44 groups were significantly lower than that of P32 and P36 groups. PPV of P44 group was significantly lower than that of P32 and P36 groups, but not different from that of P40 group (P > 0.05).

The Chi-square test results are shown in Table 4. The Chi² value (28.3–32.7) was greater than Chi²(0.05) (27.6) for the logistic model. Based on this evaluation criterion, the logistic model did not fit the data well for all groups, and this model was not used to compare in following steps. Regardless of the protein level, the Chi² values for the Richards model (5.37–5.89) were less than those for the Gompertz model (8.15–12.44) and von Bertalanffy model (14.4–22.2).

The predicted values of the fitting parameters of the Gompertz, von Bertalanffy and Richards models of body weight in the gibel carp fed diets at the four protein levels are shown in Table 5. The fitted parameters of the three models showed that, regardless of the protein level, the predicted maximum body weight (A) from the Gompertz model (348–423 g) and von Bertalanffy model (366–403 g) were lower than that from the Richards model (472–622 g). For all three models, the values of A, the weight corresponding to the inflection point and the age in weeks corresponding to the inflection point were substantially greater (29.5–33.1 wk) for the P40 diet than for the P32, P36 and P44 diets.

The observed and model-based predicted values of body weight during the 41-wk study of the gibel carp fed four diets are

| Table 2 | The four nonlinear growth mathematics models utilized in gibel carp. |
|------------------|--------------------------|
| Item             | Gompertz Equation       | Logistic Equation          | Von Bertalanffy Equation | Richards Equation  |
| Expression       | $Y_t = Ae^{Be^{Ckt}}$   | $Y_t = A/\left(1 + Be^{Ckt}\right)$ | $Y_t = A/\left(1 - e^{-kt}\right)$ | $Y_t = A/\left(1 - Be^{Ckt}\right)$ |
| AGR, g/wk        | $dy/dt = kBe^{kt} e^{Be^{kt}}$ | $dy/dt = kBe^{kt}/\left(1 + Be^{kt}\right)^2$ | $dy/dt = 3ke^{kt}/\left(1 + e^{kt}\right)^2$ | $dy/dt = 3ke^{kt}/\left(1 - Be^{kt}\right)^2$ |
| BWI, g           | $A/e^{kt}$             | $2A/27$                    | $18A/27/27$              | $18A/27/27$               |
| WI               | $\left(\ln\theta\right)/\left(\ln\theta\right)$ | $\left(\ln\theta\right)/\left(\ln\theta\right)$ | $\left(\ln\theta\right)/\left(\ln\theta\right)$ | $\left(\ln\theta\right)/\left(\ln\theta\right)$ |

$Y_t$—weight of the age of t wk; $A$—asymptotic average weight; $B$—integration constant; $k$—instantaneous relative growth rate coefficient; $t$—weekly age. AGR—absolute growth rate; BWI—body weight at inflection point; WI—weeks of inflection.

| Table 3 | Effects of protein levels on growth performance of gibel carp (Means ± SED). |
|------------------|--------------------------|
| Performance      | P32 | P36 | P40 | P44 |
| Final weight, g  | $222 ± 14.4^a$ | $231 ± 16.3^{ab}$ | $242 ± 4.3^{ab}$ | $236 ± 4.9^{ab}$ |
| SGR, %/d         | 1.68 ± 0.02 | 1.69 ± 0.02 | 1.71 ± 0.00 | 1.70 ± 0.01 |
| FF, %/d          | 1.50 ± 0.07^c | 1.39 ± 0.06^b | 1.31 ± 0.03^{ab} | 1.26 ± 0.07^c |
| FCR               | 2.16 ± 1.0^c | 2.01 ± 0.09^c | 1.90 ± 0.05^{ab} | 1.82 ± 0.10^c |
| PPV, %            | 23.9 ± 2.9^b | 23.2 ± 6.1^b | 22.4 ± 3.4^{ab} | 20.9 ± 6.1^b |

Groups of P32, P36, P40 and P44 denoted dietary protein levels, 32, 36, 40 and 44 g/kg, respectively. 

Table 4 Chi-square results of measured and estimated values of Gompertz, logistic, von Bertalanffy and Richards models.

| Model     | P32 | P36 | P40 | P44 |
|-----------|-----|-----|-----|-----|
| Gompertz  | 8.15 | 9.41 | 9.94 | 12.4 |
| Logistic  | 28.3 | 30.0 | 29.5 | 32.7 |
| von Bertalanffy | 15.6 | 14.4 | 22.2 | 18.9 |
| Richards  | 5.37 | 4.67 | 4.12 | 5.89 |

Chi²(0.05) = 27.6 (df = 17). Groups of P32, P36, P40 and P44 denoted dietary protein levels, 32, 36, 40 and 44 g/kg, respectively.
presented in Table 6 (same in Fig. 1) and Table 7, respectively. Regardless of the protein levels, the S-shaped–like curves were fitted to the growth trend (Fig. 1). According to the ANOVA, four growth stages of gibel carp during 0–41 wk were shown obviously (Table 6). Before the 9th week, fish fed diets P40 and P44 showed significantly higher body weight than that of fish fed low protein diets. The body weight of fish fed diet P36 was not different from that of P40 group during the 9th to 22nd week, while P40 and P44 groups still showed the highest growth and significantly higher than that of P32 group. During 24–32 wk, body weights of all groups were not significantly different. However, in 34–41 wk, fish fed diet P40 showed the highest body weight, and during the whole experiment period, fish fed low protein diet P32 showed the lowest performance. The growth curves of the gibel carp were fitted well by the three models at the four protein levels, with all the R² values above 0.990. However, the corresponding predicted values based on the Richards model generally was found to be the best matched to the observed values as shown in Table 7 with the lowest RSS and RV, and then for Gompertz and least for von Bertalanffy models.

Fig. 2 shows the AGR (formula shown in Table 2) based on the Gompertz, Richards, and von Bertalanffy models of the gibel carp fed the diets with four protein levels. Regardless of the protein levels, the WI of the Richards model was at least one week later than that of the Gompertz and four weeks later than that of the von Bertalanffy model. Beyond the inflection point, the growth rate decreased for the four protein levels in all three models. Based on the three models, the fish fed the P40 diet had the highest AGR, while the lowest for fish of P32 (Fig. 2). This result showed that in the present study, the optimal protein level for the gibel carp based on the cumulative growth curves for body weight was 400 mg/kg, which was in accordance with the results of FCR and PPV.

### 4. Discussion

The logistic curve is an adequate description of the laboratory grown organisms with simple life cycles (Hernandez-Llamas and Ratkowsky, 2004), and is not recommended in fisheries and aquaculture to describe fish growth (Krebs, 1994). This model is not fit well for gibel carp in the present study. The von Bertalanffy model may be the most prevalent growth model for fish in the literature (Ricker, 1979; He and Stewart, 2002; Beatriz and Dalila, 1996; Camito, 1998), but it is not always the “best” model and some have been argued that it is an inappropriate model (Knight, 1968; Von Rosen, 1991). For example, Katsanevakis and Maravellas (2008) found that the von Bertalanffy model might be the “best” model in only about one-third of the 133 length-at-age data sets examined, when compared to the Gompertz, logistic and a power model. In addition, the Gompertz model is widely used in place of the von Bertalanffy model when modeling growth during larval or early life stage (Ricker, 1979). The wild-caught crucian carp of age 1–18 years fit well in von Bertalanffy model with asymptotic weight at 832.3 g and age of inflection at 5–6 yr (Jiang, 1996). However, the artificial genotype modified gibel carp has higher growth rate than wild crucian carp in early life, and most pond cultured gibel carp are harvested in 2–3yr old with body weight about 500 g as similar size as predicted growth performance in the present study with Richards and Gompertz models, which
Richards model was the best and live weight at maximal growth phase. In the present study, the various traits such as live weight, weight gain, rate of maturity, age...

Table 7

| Time, wk | P32 | P36 | P40 | P44 |
|---------|-----|-----|-----|-----|
|         | Gompertz | Von Bertalanffy | Richards | Gompertz | Von Bertalanffy | Richards | Gompertz | Von Bertalanffy | Richards | Gompertz | Von Bertalanffy | Richards |
| 0       | 3.05 | 0.00 | 0.66 | 3.62 | 0.00 | 1.00 | 4.18 | 0.00 | 1.48 | 4.46 | 0.00 | 1.66 |
| 1       | 3.69 | 0.79 | 3.70 | 7.44 | 0.87 | 4.58 | 8.14 | 0.82 | 5.38 | 8.61 | 0.83 | 5.81 |
| 2       | 10.00 | 3.21 | 7.55 | 11.3 | 3.49 | 8.83 | 12.0 | 3.35 | 9.69 | 12.6 | 3.36 | 10.3 |
| 3       | 14.88 | 7.71 | 13.0 | 16.4 | 8.35 | 14.6 | 17.1 | 8.07 | 15.4 | 17.9 | 8.08 | 16.3 |
| 4       | 20.90 | 14.44 | 20.0 | 22.8 | 15.5 | 22.0 | 23.4 | 15.1 | 22.6 | 24.4 | 15.1 | 23.6 |
| 5       | 28.41 | 23.1 | 28.3 | 30.7 | 24.8 | 30.7 | 31.2 | 24.3 | 31.2 | 32.3 | 24.3 | 32.4 |
| 6       | 37.37 | 33.7 | 38.0 | 40.0 | 36.0 | 40.8 | 40.3 | 35.4 | 41.6 | 41.6 | 35.4 | 42.3 |
| 7       | 47.67 | 45.7 | 48.8 | 50.7 | 48.7 | 51.9 | 50.9 | 48.2 | 52.1 | 52.1 | 48.0 | 53.4 |
| 8       | 65.44 | 65.8 | 66.8 | 68.9 | 69.8 | 70.4 | 69.0 | 69.7 | 70.6 | 70.3 | 69.3 | 71.8 |

R² = the coefficient of determination; RSS = residual sum of squares; RV = residual variance.

Groups of P32, P36, P40 and P44 denoted dietary protein levels, 32, 36, 40 and 44 g/kg, respectively.

much “better” fitted than von Bertalanffy model. The Richards model is known as the two-phase growth model, which generalized and modified from the von Bertalanffy model. The Richards model has a more flexible function with a variable inflection point that provides a more complete description of growth process in a variety of animal species (Goonewardene et al., 2003; Hernandez-Llamas and Ratkowsky, 2004; Katsanevakis and Maravelias, 2008). An appropriate growth model has economic importance of different species, different food sources and sexually mature or immature life stages. In the present study, it was shown that dietary protein level affected not only the asymptotic average weight, but also the instantaneous relative growth rate coefficient (k), which could be used as maturing index for animal (Ersoy, 2006). The optimal dietary protein level (400 mg/kg) induced lower maturing index, and accordingly delayed the WI for gibel carp. Furthermore, for all fitting models, the A value, BWI and WI for the P40 group were substantially greater than the corresponding parameters of the P32, P36 and P44 treatments. Based on the observed and predicted growth values by the best fitted Richards model, the fish fed the P40 diet had the highest AGR for all the protein levels tested. Hence, the gibel carp fed the diet with the optimal protein level could get longer stage of fast growth and improve their AGR under the similar conditions.

The dietary protein requirement of a species is of prime importance in aquaculture because feed protein influences the growth of the fish and determines the cost of feeding (Qian, 2001). Very often, practical diets are formulated to quantify the protein requirement of different fishes, such as common carp (Cyprinus carpio L.) (Cho et al., 2001), Nile tilapia (Oreochromis niloticus) (El-Sayed et al., 2003), hybrid catfish (Giri et al., 2003), and channel catfish (Ictalurus punctatus) (Li, 2008). All these studies gave out the requirement of the specific stages of fish species, such as juvenile or brood stock by a short-term growth trial. Qian (2001) reported a large variety of protein requirement (35–48.6%) by different regression models for juvenile gibel carp (4.78–24.1 g). Until now, there were no any reports on effect of dietary nutrients density on long-term growth curves and nutrients requirement of fish. In the present study, we clearly found four growth stages of gibel carp during 0–41 wk, and showed decreased dependence on dietary protein level with the growth duration of fish (Table 6). During the whole experiment period, fish fed low protein diet P32 showed the lowest, while P40 showed the highest performance. Combined with the results of FCR and PPV of the gibel carp, P40 group showed the highest biological and economic performance in the present study.
5. Conclusion

The analysis indicated that 1) the Richards model was the best predictive model for the growth of the gibel carp fed various dietary protein level diets; 2) according to the optimal Richards model for the gibel carp at the optimal (400 g/kg) protein level, the growth process generally consisted of a fast growth phase during 0–34 wk with a predicted weight from 1.48 to 191 g, and a slow growth phase after wk 34 to the predicted asymptotic weight of 622 g.

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