



Original research article

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 \pm 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 ($P \geq 0.05$). The growth response of the gibel carp for each group was the best fitted by Richards model with the lowest χ^2 , 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 χ^2 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 Ratkowsky, 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

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gynocercous subspecies of goldfish or crucian carp, and the production of 2012 has exceeded 2 million tons in China (MOA-China, 2012). The increased use of low-cost ingredients as a substitute for fish meal in the feed for gibel carp has increased the likelihood that the level of digestible nutrients, such as protein and essential amino acids, will decrease. This change might affect the growth and health of the cultured fish 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; Luo et al., 2004; Xue et al., 2012). This process may be detrimental to fish growth. Therefore, the knowledge of the protein requirement of the 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.

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 carbohydrate. 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) profile (EAA/CP), and named as P32, P36, P40 and P44, respectively (Table 1). Fish oil, soybean oil and lecithin were used to meet the fatty acids requirement of gibel carp. Porcine liver meal and brewer's yeast were used to keep the palatability and digestibility of each diet.

All ingredients were finely ground by an ultrafine grinder (SWFL50E, MUYANG Group, Jiangsu, China) and passed through 246- μ m sieve before being pelletized. The diets were made into dry pelleted sinking diets of different sizes (diameter: 0.5, 1.0, 2.0, and 3.5 mm) with a commercial pelleter (MYZL180, MUYANG Group, Jiangsu, China). All diets were stored in a freezer at -20°C during the feeding trial.

Table 1
Ingredients and nutrient composition of the experimental diets, g/kg.

Item	P32	P36	P40	P44
Ingredients¹				
Fish meal	210	250	280	339
Soybean meal	180	180	180	180
Full-fat extruded soybean	55	50	40	30
Porcine liver meal	25	25	25	25
Brewer's yeast	50	50	50	50
Wheat flour	165	165	165	165
Wheat middling	152	152	152	99
Spry-dried blood meal	0	14.7	36.7	53.7
Lecithin	5	5	5	5
Fish oil	10	10	10	10
Soybean oil	10	10	10	10
α -cellulose	105	55	13	0
Ca(H ₂ PO ₄) ₂	20	20	20	20
Vitamin and mineral premix ²	10	10	10	10
Analyzed chemical composition				
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.

CP = crude protein.

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 Beijing Enhalar 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 K₃ 4; thiamin 6; riboflavin 8; pyridoxine hydrochloride 14; vitamin B₁₂ (1%) 0.1; L-ascorbyl-2-monophosphate-Na 600; calcium pantothenate 100; amine nicotinic acid 80; biotin (2%) 0.2; folic acid 2; inositol 200; choline chloride (50%) 3,000; wheat middling 1,648; 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; zeolite 1,663.

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 (Enhalar 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 complied with Laboratory Animal Welfare Guidelines of China (Decree No. 2

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 (Statsoft Inc., Tulsa, OK, USA). The same software was used to estimate the optimal values of the parameters asymptotic average weight (A), integration constant (B), and instantaneous relative growth rate coefficient (k) for the body weight data to establish and evaluate the growth models according to the coefficient of determination (R^2), residual sum of squares (RSS), residual variance (RV) and Chi^2 were also used as complementary criteria for model performance. Chi^2 was measured according to the following equation:

$$\text{Chi}^2 = \sum_i \left(\frac{(O_i - E_i)^2}{E_i} \right),$$

where O_i = observed value at moment i , E_i = model-based predicted value at moment i .

If $\text{Chi}^2 > \text{Chi}_{0.05}^2$ ($P < 0.05$), the equation does not furnish an adequate fit to the data because the predicted value is not consistent with the observed value. If $\text{Chi}^2 < \text{Chi}_{0.05}^2$ ($P > 0.05$), the equation fits well, and the predicted value is consistent with the observed value (Yang et al., 2006). The degree of freedom of the Chi^2 goodness-of-fit test was $N - n - 1$, where N is the observed number of data points, and n is the number of fitted parameters in the model.

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

Table 3
Effects of protein levels on growth performance of gibel carp (Means \pm SED).

Performance	P32	P36	P40	P44
Final weight, g	222 \pm 14.4 ^a	231 \pm 16.3 ^{ab}	242 \pm 4.3 ^b	236 \pm 4.9 ^{ab}
SGR ¹ , %/d	1.68 \pm 0.02	1.69 \pm 0.02	1.71 \pm 0.00	1.70 \pm 0.01
FI ² , %/d	1.50 \pm 0.07 ^c	1.39 \pm 0.06 ^b	1.31 \pm 0.03 ^{ab}	1.26 \pm 0.07 ^a
FCR ³	2.16 \pm 0.10 ^c	2.01 \pm 0.09 ^b	1.90 \pm 0.05 ^{ab}	1.82 \pm 0.10 ^a
PPV ⁴ , %	23.9 \pm 0.29 ^b	23.2 \pm 0.61 ^b	22.4 \pm 0.34 ^{ab}	20.9 \pm 0.61 ^a

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

^{a,b,c}Different superscripts within a line indicate significant differences between treatments with $P < 0.05$.

¹ SGR, Specific growth rate (%/d) = $100 \times [\text{Ln}(\text{final body weight}) - \text{Ln}(\text{initial body weight})] / \text{days}$.

² FI, feed intake (%/d) = $100 \times \text{total amount of the feed consumed} / [\text{days} \times (\text{initial body weight} + \text{final body weight}) / 2]$.

³ FCR, feed conversion ratio = $\text{total amount of the feed consumed} / (\text{final body weight} - \text{initial body weight})$.

⁴ PPV, productive protein value (%) = $100 \times (\text{whole-body protein gain} / \text{protein consumption})$.

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

$\text{Chi}_{0.05}^2 = 27.6$ (df = 17).

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

levels. FCR of P40 and P44 groups were significantly lower than those 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 Chi^2 value (28.3–32.7) was greater than $\text{Chi}_{0.05}^2$ (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 Chi^2 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	Logistic	Von Bertalanffy	Richards
Expression	$Y_t = Ae^{B \exp(-kt)}$	$Y_t = A/(1 + Be^{-kt})$	$Y_t = A(1 - e^{-kt})^3$	$Y_t = A(1 - Be^{-kt})^3$
AGR, g/wk	$dy/dt = kABe^{-kt} e^{-B \exp(-kt)}$	$dy/dt = kABe^{-kt}/(1 + Be^{-kt})^2$	$dy/dt = 3kAe^{-kt}(1 - e^{-kt})^2$	$dy/dt = 3kA(1 - Be^{-kt})^2 e^{-kt}$
BWI, g	A/e	$A/2$	$8A/27$	$8A/27$
WI	$(\ln B)/k$	$(\ln B)/k$	$\ln 3/k$	$(\ln 3B)/k$

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 5
Fitting parameters of the Gompertz, Von Bertalanffy and Richards growth models in gibel carp.

Group	A, g	B	k, per wk	BWI, g	WI
Gompertz					
P32	348	4.78	0.058	128	26.9
P36	363	4.61	0.057	133	27.0
P40	423	4.62	0.052	156	29.5
P44	405	4.51	0.053	149	28.7
von Bertalanffy					
P32	366		0.046	108	23.8
P36	363		0.048	107	23.0
P40	403		0.0453	119	24.3
P44	390		0.046	116	24.0
Richards					
P32	472	0.89	0.035	140	28.4
P36	491	0.87	0.034	146	28.5
P40	622	0.86	0.029	184	32.9
P44	580	0.86	0.030	172	31.7

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

A = asymptotic average weight; k = instantaneous relative growth rate coefficient; B = integration constant; BWI = body weight at inflection point; WI = weeks of inflection.

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 feed 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

Table 6
The observed body weights (g, mean ± SD) of the gibel carp at various sampling time points.

Time, wk	P32	P36	P40	P44
0	1.85 ± 0.01	1.85 ± 0.01	1.85 ± 0.01	1.85 ± 0.01
3	3.24 ± 0.04 ^a	3.63 ± 0.03 ^b	3.70 ± 0.07 ^b	3.71 ± 0.10 ^b
5	6.28 ± 0.03 ^a	7.11 ± 0.14 ^b	7.51 ± 0.17 ^c	7.58 ± 0.14 ^c
7	11.0 ± 0.06 ^a	12.3 ± 0.18 ^b	13.0 ± 0.29 ^c	13.3 ± 0.22 ^c
9	17.4 ± 0.17 ^a	19.5 ± 0.20 ^b	20.6 ± 0.34 ^c	21.0 ± 0.42 ^c
11	26.2 ± 0.25 ^a	28.8 ± 0.39 ^b	30.1 ± 0.45 ^{bc}	31.3 ± 0.88 ^c
13	37.5 ± 0.35 ^a	40.8 ± 0.81 ^b	41.4 ± 0.90 ^b	43.2 ± 1.78 ^b
15	49.9 ± 0.54 ^a	52.9 ± 0.86 ^{bc}	54.1 ± 1.82 ^{bc}	55.5 ± 2.47 ^c
18	66.9 ± 0.77	70.5 ± 0.82	70.5 ± 3.28	72.9 ± 3.47
20	84.7 ± 0.93 ^a	88.9 ± 1.13 ^{bc}	91.2 ± 2.48 ^c	93.5 ± 3.06 ^c
22	99.2 ± 1.29 ^a	104 ± 1.33 ^{bc}	105 ± 2.40 ^{bc}	107 ± 3.80 ^c
24	110 ± 1.23	115 ± 1.34	117 ± 2.63	113 ± 4.96
26	122 ± 1.33	127 ± 1.88	127 ± 2.74	123 ± 5.41
28	135 ± 1.88	140 ± 2.63	142 ± 3.70	135 ± 6.22
30	145 ± 1.76	151 ± 2.92	154 ± 4.20	147 ± 7.64
32	155 ± 2.37	158 ± 5.11	165 ± 4.11	154 ± 8.60
34	173 ± 3.64 ^a	179 ± 4.23 ^{ab}	187 ± 3.16 ^b	185 ± 3.08 ^b
36	198 ± 4.37 ^a	201 ± 4.68 ^{ab}	214 ± 3.82 ^b	214 ± 3.16 ^b
38	211 ± 5.27 ^a	219 ± 5.57 ^{ab}	229 ± 2.97 ^b	228 ± 0.72 ^b
40	223 ± 5.99 ^a	229 ± 6.11 ^{ab}	241 ± 2.44 ^b	238 ± 2.08 ^{ab}
41	226 ± 6.44 ^a	231 ± 7.27 ^{ab}	242 ± 2.16 ^b	236 ± 2.48 ^{ab}

^{a,b,c}Different superscripts within a line indicate significant differences between treatments with P < 0.05.

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

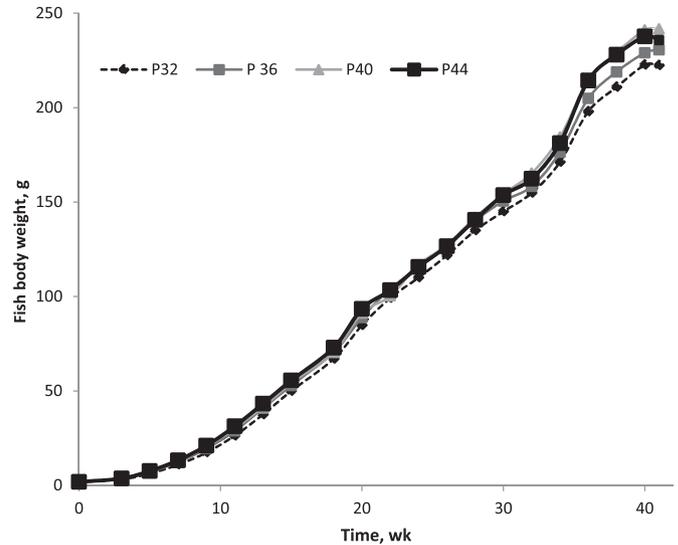


Fig. 1. Cumulate growth curves expressed as body weight of gibel carp during the experiment fed four diets with various protein levels (P32, P36, P40 and P44). Groups of P32, P36, P40 and P44 denoted dietary protein levels, 32, 36, 40 and 44 g/kg, respectively.

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; Gamito, 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 Maravelias (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 5–6yr (Jiang, 1996). However, the artificial genotype modified gibe 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

Table 7
The predicted weight (g) based on the Gompertz, von Bertalanffy and Richards models of the gibel carp at various sampling time point, with coefficient of determination, residual sum of squares and residual variance (mean \pm SE) obtained from regression analyses.

Time, wk	P32			P36			P40			P44		
	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
3	6.49	0.79	3.70	7.44	0.87	4.58	8.14	0.82	5.38	8.61	0.83	5.81
5	10.0	3.21	7.55	11.3	3.49	8.83	12.0	3.35	9.69	12.6	3.36	10.3
7	14.8	7.71	13.0	16.4	8.35	14.6	17.1	8.07	15.4	17.9	8.08	16.3
9	20.9	14.4	20.0	22.8	15.5	22.0	23.4	15.1	22.6	24.4	15.1	23.6
11	28.4	23.1	28.3	30.7	24.8	30.7	31.2	24.3	31.2	32.3	24.3	32.4
13	37.3	33.7	38.0	40.0	36.0	40.8	40.3	35.4	41.1	41.6	35.4	42.3
15	47.6	45.7	48.8	50.7	48.7	51.9	50.9	48.2	52.1	52.1	48.0	53.4
18	65.4	65.8	66.8	68.9	69.8	70.4	69.0	69.7	70.6	70.3	69.3	71.8
20	78.5	80.2	79.8	82.4	84.7	83.7	82.6	85.1	83.9	83.7	84.5	85.1
22	92.3	95.0	93.3	96.5	100	97.5	97.0	101	98.1	98.0	100	99.0
24	107	110	107	111	115	111	112	117	113	113	116	113
26	121	125	121	126.3	130	126	128	133	128	128	132	128
28	136	140	136	141	146	141	144	149	143	144	148	143
30	151	154	150	156	160	155	160	165	159	159	163	159
32	165	168	164	171	174	170	176	180	175	175	179	174
34	179	182	178	186	188	184	192	195	191	190	192	189
36	193	195	192	199	201	199	208	209	207	205	206	204
38	206	207	206	213	213	212	223	223	222	220	219	219
40	218	219	219	225	225	226	237	236	238	233	232	234
41	224	224	225	231	230	232	244	242	246	240	238	241
R ²	0.996	0.997	0.996	0.996	0.997	0.996	0.996	0.995	0.996	0.995	0.991	0.990
RSS	316	352	213	343	425	241	424	622	317	766	1138	635
RV	3.47 \pm 1.99	3.65 \pm 2.13	2.60 \pm 2.03	3.57 \pm 2.16	3.84 \pm 2.41	2.76 \pm 2.17	3.82 \pm 2.42	4.67 \pm 2.86	3.03 \pm 2.49	5.04 \pm 3.41	6.40 \pm 3.72	4.47 \pm 3.52

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 various traits such as live weight, weight gain, rate of maturity, age and live weight at maximal growth phase. In the present study, the Richards model was the best-fitting predictive growth model for the gibel carp under the present experimental conditions according to both Chi-square and RV values, while Gompertz model and von Bertalanffy model could be but not “so-good” fitting the growth curve of gibel carp. The predicted asymptotic average weight (A value: 472.1–621.6 g, when fed with various protein level diets) by the Richards model were most close to the harvest size of gibel carp in practical production (MOA-China, 2012).

In the present study, the curve describing the growth in live weight with age is S-shaped (Fig. 1). The present study demonstrated that the gibel carp is a neotenic species, with sexual maturity at an average weight of 200 g (Gui and Zhou, 2010). The weight gain decreased after the gibel carp attained sexual maturity, which was in accordance with most fish species (He and Stewart, 2002; Jone, 2000). However, the growth curves of Atlantic halibut reared in larger tanks and fed frozen capelin and herring for 3 years approached a linear relationship (Björnsson, 1995). In that study, the female Atlantic halibut (final body weight 12 kg) were immature, whereas the male Atlantic halibut (final body weight 7 kg) matured sexually during the experimental period; in this species, females and males generally become sexually mature at an average weight of 12.7 and 3.2 kg, respectively (Björnsson, 1995). The growth curves of pigs showed that weight gain increased rapidly in the early part of the growth period and then approached a plateau when the pigs reached sexual maturity (Strathe et al., 2009). The differences among these growth curves can be attributed to the use

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.

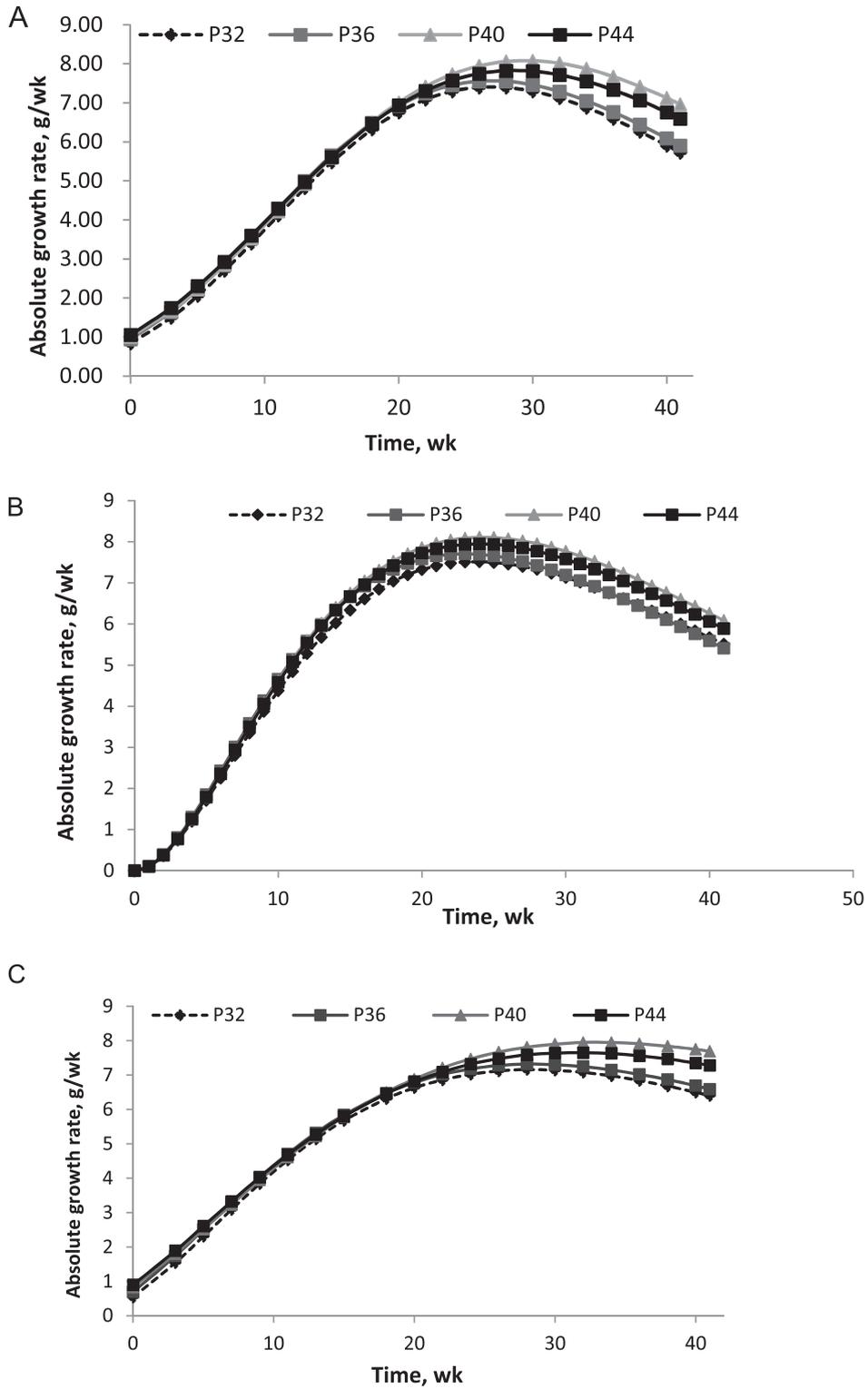


Fig. 2. Absolute growth rates of gibel carp fed diets at four protein levels based on Gompertz model (A), von Bertalanffy model (B) and Richards model (C). Groups of P32, P36, P40 and P44 denoted dietary protein levels, 32, 36, 40 and 44 g/kg, respectively.

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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