Postprint: Dietary Protein Requirement of Allogynogenetic Gibel Carp (Carassius auratus gibelio) after Long-term Starvation

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Abstract

To investigate the dietary protein requirement level and adaptive strategies of juvenile gibel carp (Carassius auratus gibelio) after long-term starvation, this experiment first subjected juvenile gibel carp [(11.6±0.4) g] to continuous starvation for 90 days at a water temperature of (15.2±2.0) °C, followed by refeeding for 56 days with diets containing 20% (P20), 25% (P25), 30% (P30), 35% (P35), and 40% (P40) protein at a water temperature of (24.4±2.2) °C. Changes in morphological indices, organ coefficients, body composition, and intestinal digestive enzyme activities were monitored during starvation and refeeding. The results showed: 1) After 90 days of starvation, fish body weight decreased significantly ($P<0.05$), hepatosomatic index and intestinal length coefficient decreased significantly ($P<0.05$), intestinal protease, amylase, and lipase activities decreased significantly ($P<0.05$), body moisture and crude ash contents increased significantly ($P<0.05$), and crude lipid and crude protein contents decreased significantly ($P<0.05$). 2) After refeeding, on day 14, there were no significant differences in body weight among groups ($P>0.05$); on day 28, the P20 group exhibited significantly lower body weight than the P30, P35, and P40 groups ($P<0.05$), and on days 42 and 56, the P35 and P40 groups showed significantly higher body weight than other groups ($P<0.05$), with these two groups displaying similar specific growth rates and feed conversion ratios. 3) On day 56 of refeeding, the crude protein content in the P20 group was significantly lower than other groups ($P<0.05$), the crude lipid content in the P20 and P25 groups was significantly lower than other groups ($P<0.05$), the crude ash content in the P20 and P25 groups was significantly higher than other groups ($P<0.05$), and the body moisture content in the P20 and P25 groups was significantly higher than in the P35 and P40 groups ($P<0.05$). 4) After refeeding, intestinal digestive enzyme activities gradually increased; protease activity increased with dietary protein level, being significantly higher in the P35 and P40 groups on

day 28 (P<0.05) and significantly higher in the P40 group on day 56 (P<0.05); amylase activity decreased with dietary protein level, being significantly lower in the P35 and P40 groups on day 28 (P<0.05) and significantly lower in the P30, P35, and P40 groups on day 56 (P<0.05). These results suggest that juvenile gibel carp exhibit substantial differences in utilization and adaptation to diets with different protein levels after long-term starvation; during the initial refeeding stage, dietary protein level had little effect on growth, but after feeding and digestive functions gradually recovered, higher protein levels (35%~40%) effectively promoted growth, with regression analysis yielding a theoretical optimal dietary protein level of 38.1%.

Full Text

Requirement of Dietary Protein in Gibel Carp (Carassius auratus gibelio) Following Long-Term Starvation

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Abstract

This study investigated the dietary protein requirement and adaptive strategies of juvenile gibel carp (Carassius auratus gibelio) following long-term starvation. Juvenile gibel carp with an initial weight of (11.6±0.4) g were subjected to a 90-day starvation period at a water temperature of (15.2±2.0) ℃, followed by a 56-day re-feeding trial with diets containing 20% (P20), 25% (P25), 30% (P30), 35% (P35), or 40% (P40) crude protein at (24.4±2.2) ℃. Body measurements, viscera indices, body composition, and intestinal digestive enzyme activities were monitored throughout both periods. The results showed: (1) After 90 days of starvation, body weight, liver index, and intestinal tract length index decreased significantly (P<0.05), intestinal protease, amylase, and lipase activities declined markedly (P<0.05), while moisture and ash content increased significantly (P<0.05) and crude protein and lipid content decreased significantly (P<0.05). (2) During re-feeding, no significant differences in body weight were observed among groups on day 14 (P>0.05). On day 28, the P20 group exhibited significantly lower body weight than the P30, P35, and P40 groups (P<0.05). By days 42 and 56, the P35 and P40 groups showed significantly higher body weight than other groups (P<0.05), with similar specific growth rates (SGR) and feed conversion ratios (FCR) between these two groups. (3) On day 56 of re-feeding, the P20 group had significantly lower body crude protein content than other groups (P<0.05), while the P20 and P25 groups showed significantly lower crude lipid content (P<0.05) and higher ash content (P<0.05) compared to other groups. Moisture content in the P20 and P25 groups was significantly higher than in the P35 and P40 groups (P<0.05). (4) Following re-feeding, in-

testinal digestive enzyme activities gradually increased. Protease activity rose with increasing dietary protein level, with the P35 and P40 groups showing significantly higher activity than other groups on day 28 ($P<0.05$), and the P40 group significantly higher than others on day 56 ($P<0.05$). Amylase activity decreased with increasing dietary protein level, with the P35 and P40 groups significantly lower than other groups on day 28 ($P<0.05$), and the P30, P35, and P40 groups significantly lower on day 56 ($P<0.05$). These findings indicate that juvenile gibel carp exhibit differential utilization and adaptation to varying dietary protein levels after long-term starvation. While dietary protein level has limited impact on growth during the initial re-feeding phase, higher protein levels (35%-40%) effectively promote growth once feeding and digestive functions are restored. Polynomial regression analysis identified the theoretical optimal dietary protein level as 38.1%.

Keywords: Gibel carp (Carassius auratus gibelio); long-term starvation; dietary protein level; specific growth rate; feed conversion ratio

Introduction

Environmental conditions directly affect animal growth and survival. During extreme conditions such as food scarcity, organisms continuously deplete energy and material reserves, leading to reduced activity and metabolic levels, compromised immunity, and even mortality. Simultaneously, animals exhibit adaptive responses in behavior, physiological structure, and intestinal environment and function. During initial starvation, organisms typically maintain relatively high activity and metabolic levels to respond promptly when food becomes available or when facing other environmental hazards. However, after prolonged starvation, physiological activity and metabolism are generally maintained at lower levels to minimize energy and material consumption, preserve body weight, and extend survival. Fish naturally experience starvation risks during growth, and long-term starvation alters swimming ability, feeding behavior, digestive and absorptive functions, and metabolic levels. These changes represent adaptive responses formed to maximize starvation tolerance and maintain survival. Fish possess strong starvation resistance, and growth can gradually resume after re-feeding. Short-term starvation may even induce compensatory growth upon re-feeding. However, fish experiencing long-term starvation may exhibit altered nutrient adaptation, utilization capacity, and actual requirements compared to optimal levels under normal culture conditions.

In aquaculture practice, starved populations, such as those after overwintering, are often fed high-protein diets or increased feeding frequency to achieve rapid recovery. However, excessive protein levels may impose greater metabolic stress on starved fish, failing to achieve the goal of rapidly restoring feeding, digestive, and metabolic functions. Gibel carp (Carassius auratus gibelio) is a major freshwater aquaculture species in China, with extensive research conducted and

applied on nutritional requirements during larval, juvenile, and adult stages. This study continuously monitored morphological indices, viscera coefficients, body composition, and intestinal digestive enzyme activities in juvenile gibel carp during prolonged starvation, and compared changes in these indices after re-feeding with different protein levels. The objectives were to understand the effects of long-term starvation on morphology and physiology of juvenile gibel carp, determine actual protein requirements after starvation stress, evaluate adaptation capacity to dietary protein levels, and provide references for feeding strategies in aquaculture production.

Materials and Methods

1.1 Experimental Fish Juvenile gibel carp used in this experiment were bred at the aquaculture base of the Institute of Fisheries, Anhui Academy of Agricultural Sciences. Prior to the experiment, fish were transferred to an indoor recirculating aquaculture system with 300 L tanks. Initial body length was (7.5±0.3) cm and initial body weight was (11.6±0.4) g.

1.2 Experimental Diets Five isoenergetic and isolipidic experimental diets with crude protein levels of 20%, 25%, 30%, 35%, and 40% were formulated for the re-feeding trial, designated as P20, P25, P30, P35, and P40, respectively. Casein was used to adjust protein levels, and dextrin to adjust energy levels. Diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of the experimental diets (air-dry basis)

Items	Diets
Ingredients	
Fishmeal	
Soybean meal	
Rapeseed meal	
Casein	
Soybean oil	
Dextrin	
Cellulose	
Vitamin premix[1]	
Mineral premix[2]	
Choline chloride	
Sodium carboxymethyl cellulose (CMC)	
Total	
Nutrient levels	
Crude protein	
Crude lipid	

Items	Diets
Crude fiber	
Ash	

[1] Vitamin premix provided the following per kg of diet: VB1 20 mg, VB2 20 mg, VB6 20 mg, VB12 0.020 mg, folic acid 5 mg, calcium pantothenate 50 mg, inositol 100 mg, niacin 100 mg, biotin 0.1 mg, VC 100 mg, VA 6,000 IU, VD 2,000 IU, VE 50 mg, VK 10 mg, starch 23 mg.

[2] Mineral premix provided the following per kg of diet: NaCl 500 mg, $MgSO4 \cdot 7H2O$ 8,155.6 mg, $NaH2PO4 \cdot 2H2O$ 12,500 mg, KH2PO4 16,000 mg, $Ca(H2PO4)2 \cdot H2O$ 7,650.6 mg, $FeSO4 \cdot 7H2O$ 2,286.2 mg, $C6H10CaO6 \cdot 5H2O$ 1,750 mg, $ZnSO4 \cdot 7H2O$ 178 mg, $MnSO4 \cdot H2O$ 61.4 mg, $CuSO4 \cdot 5H2O$ 15.5 mg, $CoSO4 \cdot 7H2O$ 34.5 mg, KI 114.8 mg, starch 753.4 mg.

1.3 Starvation Procedure After transfer to the indoor system, fish were subjected to starvation for 90 days at a water temperature of (15.2±2.0) ℃. Every 30 days, body length, body weight, viscera index (VI), liver index (LI), abdominal fat index (AFI), intestinal tract weight index, and intestinal tract length index were measured. Intestinal length was measured as the length of the entire intestine when naturally extended.

1.4 Re-feeding After 90 days of starvation, similarly sized juveniles with body length of (7.4±0.2) cm and body weight of (8.4±0.6) g were randomly divided into 5 groups, each with 3 replicates of 40 fish per 300 L tank. The recirculating water exchange rate was 150 L/h, and water temperature during feeding was (24.4±2.2) ℃. Fish were fed to satiation three times daily at 08:00, 13:00, and 18:00, with each group receiving one experimental diet. Feed intake was recorded. Every 14 days, 4 fish per tank were measured for morphological indices, dissected to separate viscera, and intestinal samples were stored at -80 ℃ for digestive enzyme analysis. After 8 weeks of re-feeding, body length and weight were measured individually, and 4 fish per tank were randomly sampled for viscera indices and intestinal samples. All measurements and operations were performed after 24 h of fasting under anesthesia.

1.5 Index Analysis Protease, lipase, and -amylase activities were determined using assay kits (Nanjing Jiancheng Bioengineering Institute). Moisture content in fish and diets was determined by drying at 105 ℃ to constant weight. Crude protein content was measured by the Kjeldahl method, crude lipid by Soxhlet extraction, and ash content by incineration at 550 ℃. Morphological indices were calculated as follows:

Specific growth rate [SGR, %/d] = $100 \times (\ln Wt - \ln W0)/T$
Condition factor [CF, g/cm^3] = $100 \times Wt/Lt^3$
Feed conversion ratio [FCR] = $FI/(Wt - W0)$

Viscera index (%) = 100 × Wv/BW
Liver index (%) = 100 × Wh/BW
Abdominal fat index (%) = 100 × Wf/BW
Intestinal tract weight index (%) = 100 × Wd/BW
Intestinal tract length index (%) = 100 × Ld/L

Where: W0, Wt, and Lt represent initial body weight (g), body weight after t days of feeding (g), and body length (cm), respectively; T is the number of feeding days; FI is feed intake (dry matter basis) (g); BW, Wv, Wh, Wf, and Wd represent body weight (g), viscera weight (g), liver weight (g), abdominal fat weight (g), and intestine weight (g), respectively; L and Ld represent body length (cm) and intestine length (cm), respectively.

1.6 Data Analysis Experimental data were analyzed and plotted using SPSS 19.0 and Microsoft Excel 2010. One-way ANOVA was performed on measured and monitored data, with Duncan' s multiple range test used for inter-group significance analysis ($P<0.05$). Polynomial regression analysis was used to determine the theoretical optimal protein level.

Results

2.1 Body Measurements and Viscera Indices After Starvation As shown in Table 2 , body weight decreased significantly with starvation duration ($P<0.05$), with 9.5% loss by day 30, 18.1% by day 60, and 27.6% by day 90. Body length decreased slightly on day 60 but not significantly ($P>0.05$). Viscera index, liver index, and intestinal tract length index all decreased with starvation duration, being significantly lower on day 60 than on days 0 and 30 ($P<0.05$), and significantly lower on day 90 than at all other time points ($P<0.05$). Abdominal fat index decreased significantly with starvation duration ($P<0.05$). No significant differences in intestinal tract weight index were observed among time points ($P>0.05$). Condition factor (y) was negatively correlated with starvation time (x) ($y = -0.007x + 2.695$, $R = 0.997$).

Table 2 Variation of body measurements and viscera indices of gibel carp (Carassius auratus gibelio) during starvation

Starvation time (d)	Body length (cm)	Condition factor (g/cm³)	Abdominal fat index (%)	Intestinal tract weight index (%)	Intestinal tract length index (%)
0	7.5±0.3	2.71±0.10d	3.3±0.0c	3.5±0.1	3.2±0.0c
30	7.5±0.3	2.44±0.10c	3.2±0.1c	3.5±0.1	3.0±0.0c
60	7.4±0.2	2.22±0.11b	2.3±0.0b	3.6±0.1	2.3±0.0b
90	7.4±0.2	2.00±0.14a	1.6±0.0a	3.5±0.1	1.6±0.0a

In the same column, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), while different letters indicate significant difference (P<0.05). The same applies to Table 3, Table 4, and Table 7.

2.2 Body Measurements and Viscera Indices After Re-feeding As shown in Table 3, starved gibel carp were re-fed with different protein levels. On day 14 of re-feeding, weight gain ranged from 3.3% to 6.4% with no significant differences among groups (P>0.05). On day 28, weight gain ranged from 17.9% to 27.3%, with the P20 group showing significantly lower body weight than the P30, P35, and P40 groups (P<0.05). On day 42, weight gain ranged from 40.2% to 83.3%, with body weight increasing with dietary protein level and then plateauing. The P35 and P40 groups showed no significant difference between them (P>0.05) but were significantly higher than other groups (P<0.05). On day 56, weight gain ranged from 60.1% to 156.0%, with the same inter-group relationships as on day 42.

On day 56 of re-feeding, condition factor increased with dietary protein level, with no significant difference between P35 and P40 groups (P>0.05), but both were significantly higher than other groups (P<0.05). Within each group, condition factor was highest on day 28 of re-feeding and subsequently decreased.

Table 3 Variation of body weight and condition factor of gibel carp (Carassius auratus gibelio) after re-feeding

Groups	Day 14	Day 28	Day 42	Day 56	Day 14	Day 28	Day 42	Day 56
	Body weight (g)				**Condition factor (g/cm³)**			
P20	8.68±0.20	9.90±0.25a	11.78±0.33a	13.45±0.42a	2.44±0.10	2.60±0.22a	2.38±0.08a	2.18±0.11a
P25	8.76±0.24	10.23±0.30a	12.13±0.41b	15.03±0.25b	2.51±0.10	2.68±0.15a	2.45±0.10a	2.25±0.13a
P30	8.88±0.30	10.56±0.37b	14.85±0.24c	19.02±0.30c	2.64±0.12	2.84±0.16b	2.62±0.12b	2.46±0.10b
P35	8.94±0.36	10.72±0.22b	15.40±0.29d	21.50±0.37d	2.85±0.16	3.09±0.15c	2.90±0.11c	2.72±0.13c
P40	8.89±0.21	10.69±0.26b	16.34±0.36d	22.36±0.47d	2.79±0.14	3.24±0.20c	3.02±0.14c	2.83±0.12c

As shown in Table 4, viscera indices changed after re-feeding. Liver index increased compared to starvation values. On day 28, the P20 group was significantly lower than other groups (P<0.05), with no significant differences among other groups (P>0.05). On day 56, liver indices were higher than on day 28 in all groups, with the P40 group significantly lower than other groups (P<0.05). On day 28 of re-feeding, intestinal tract weight index was slightly lower than during starvation, with no significant differences among groups (P>0.05). On day 56, intestinal tract weight index was lower than on day 28, again with no

significant differences among groups (P>0.05). On day 28 of re-feeding, intestinal tract length index was higher than during starvation, with values on day 56 similar to day 28 and no significant differences among groups (P>0.05).

Table 4 Variation of viscera indices of gibel carp (Carassius auratus gibelio) after re-feeding

Groups	Liver index	Intestinal tract weight index		Intestinal tract length index	
	Day 28	Day 28	Day 56	Day 28	Day 56
P20	2.8±0.1a	5.4±0.2c	5.4±0.1	2.8±0.1	3.4±0.0
P25	3.1±0.1b	5.2±0.3bc	5.2±0.1	2.6±0.3	3.2±0.1
P30	3.3±0.1b	4.9±0.2b	4.9±0.1	2.6±0.2	3.3±0.0
P35	3.2±0.1b	5.0±0.2b	5.0±0.1	2.8±0.1	3.2±0.1
P40	3.2±0.1b	4.3±0.1a	4.3±0.1	2.7±0.2	3.4±0.1

2.3 SGR and FCR During Starvation and Re-feeding During the re-feeding period, SGR increased with dietary protein level, with the P35 and P40 groups showing higher values than other groups and similar values between them. SGR varied among different re-feeding periods, being higher in later stages than early stages. The highest SGR occurred during days 29-42, decreasing during days 43-56. Across all groups, SGR followed the pattern: days 1-14 < days 15-28 < days 1-56 < days 43-56 < days 29-42 (Figure 1 [Figure 1: see original paper]).

As shown in Table 5 , quadratic regression analysis between dietary protein level and SGR during different re-feeding periods revealed that the theoretical optimal protein level for maximum SGR increased with re-feeding duration before day 42, then decreased during days 43-56. For the entire re-feeding period (days 1-56), the theoretical optimal level was 40.5% based on quadratic regression, and 39.0% based on cubic regression analysis.

Figure 1 Polynomial fitting curves between dietary protein level and specific growth rate (SGR) in different re-feeding periods

Table 5 Fitting equations for dietary protein level and SGR and theoretical optimal dietary protein levels

Re-feeding time	Fitting equation of dietary protein level (x) and SGR (y)	Theoretical optimal dietary protein level (vertex x value) (%)
Days 1-14	$y = -0.00074x^2 + 0.05435x - 0.6272$	36.7
Days 15-28	$y = -0.00105x^2 + 0.08189x - 0.2793$	39.0

Re-feeding time	Fitting equation of dietary protein level (x) and SGR (y)	Theoretical optimal dietary protein level (vertex x value) (%)
Days 29-42	$y = -0.00294x^2 + 0.2576x - 2.919$	43.8
Days 43-56	$y = -0.00357x^2 + 0.2798x - 3.116$	39.2
Days 1-56	$y = -0.00008x^3 + 0.00644x^2 - 0.1545x + 1.325$	39.0

FCR was higher during early re-feeding stages than later stages, particularly high during days 1-14. Within groups, FCR values were similar between days 29-42 and days 43-56. During the same re-feeding period, FCR decreased with increasing dietary protein level, with the P35 and P40 groups showing similar and lower FCR values than other groups (Figure 2 [Figure 2: see original paper]).

As shown in Table 6 , quadratic regression analysis between dietary protein level and FCR during different re-feeding periods indicated that the theoretical optimal protein level for minimum FCR increased with re-feeding duration. For the entire re-feeding period (days 1-56), the theoretical optimal level was 44.8% based on quadratic regression, and 37.7% based on cubic regression analysis.

Figure 2 Polynomial fitting curves between dietary protein level and feed conversion ratio (FCR) in different re-feeding periods

Table 6 Polynomial fitting equations for dietary protein level and FCR and theoretical optimal dietary protein levels

Re-feeding time	Fitting equation of dietary protein level (x) and FCR (y)	Theoretical optimal dietary protein level (vertex x value) (%)
Days 1-14	$y = 0.039x^2 - 2.830x + 60.1$	36.3
Days 15-28	$y = 0.005x^2 - 0.408x + 11.87$	40.8
Days 29-42	$y = 0.005x^2 - 0.475x + 11.68$	47.5
Days 43-56	$y = 0.004x^2 - 0.434x + 11.72$	54.3
Days 1-56	$y = 0.00087x^3 - 0.07117x^2 + 1.656x - 6.132$	37.7

2.4 Body Composition After Starvation and Re-feeding As shown in Table 7 , crude protein and crude lipid content decreased significantly ($P<0.05$)

while ash and moisture content increased significantly (P<0.05) after 90 days of starvation. Compared to day 90 of starvation, all groups showed significantly higher crude protein and crude lipid content (P<0.05) and lower moisture and ash content (P<0.05) on day 56 of re-feeding. The P20 group had significantly lower body crude protein content than on day 0 of starvation (P<0.05), while other groups showed no significant difference from day 0 (P>0.05). The P20 and P25 groups had significantly lower body crude lipid content than on day 0 (P<0.05), while the P30, P35, and P40 groups showed no significant difference from day 0 (P>0.05). Body moisture content decreased with increasing dietary protein level but showed no significant difference from day 0 in any group on day 56 of re-feeding (P>0.05). The P20 and P25 groups had significantly higher ash content than other groups (P<0.05) but significantly lower than on day 90 of starvation (P<0.05), with no significant difference from day 0 (P>0.05).

Table 7 Variation of body composition of gibel carp (Carassius auratus gibelio) after starvation and re-feeding (dry matter basis)

Process	Time (d)	Groups	Moisture	Crude protein	Crude lipid	Ash
Starvation	0	-	68.9±0.4ab	57.8±0.4c	17.5±0.4c	18.8±0.2b
	90	-	71.5±0.6c	55.7±0.3a	12.1±0.3a	20.9±0.5c
Re-feeding	56	P20	70.2±0.7b	57.0±0.5b	15.4±0.4b	19.3±0.4b
		P25	69.6±0.5b	58.0±0.6c	15.9±0.3b	18.6±0.4b
		P30	69.5±0.6ab	58.7±0.5c	17.9±0.6c	17.4±0.2a
		P35	68.6±0.5a	58.6±0.7c	18.3±0.5c	16.9±0.3a
		P40	68.2±0.4a	58.4±0.6c	17.7±0.5c	17.0±0.3a

2.5 Intestinal Digestive Enzyme Activities After Starvation and Re-feeding As shown in Table 8 , intestinal protease, lipase, and amylase activities decreased significantly after 90 days of starvation (P<0.05). After re-feeding, digestive enzyme activities increased. Protease activity increased with dietary protein level, with the P35 and P40 groups significantly higher than other groups on day 28 (P<0.05), and the P40 group significantly higher than other groups on day 56 (P<0.05). Lipase activity showed no significant differences among groups (P>0.05). Amylase activity decreased with increasing dietary protein level, with the P35 and P40 groups significantly lower than other groups on day 28 (P<0.05), and the P30, P35, and P40 groups significantly lower on day 56 (P<0.05). Within the same group, protease and amylase activities were higher on day 56 than on day 28 of re-feeding.

Table 8 Variation of intestinal digestive enzyme activities of gibel carp (Carassius auratus gibelio) after starvation and re-feeding (U/(g · min))

Process	Time (d)	Groups	Protease	Lipase	Amylase
Starvation	90	-	19.2±3.9a	1.2±0.3a	2.5±0.4a
Re-feeding	28	P20	122.2±12.1a	8.2±2.2	160.2±12.0d
		P25	145.8±20.3a	8.5±0.9	145.6±10.1c
		P30	220.4±25.1b	8.9±1.1	120.7±13.4b
		P35	255.2±31.0c	8.6±0.6	90.1±11.2a
		P40	244.7±29.3c	8.8±0.7	88.8±9.2a
	56	P20	180.5±24.0a	8.6±1.0	190.7±8.2c
		P25	260.3±10.6b	9.5±2.2	160.4±11.8b
		P30	389.5±28.2c	9.3±0.3	125.5±20.0a
		P35	400.0±19.2c	8.9±1.0	120.3±17.1a
		P40	415.7±22.1d	9.2±0.4	107.7±15.4a

In different starvation time points, different groups at day 28 after re-feeding, and different groups at day 56 after re-feeding, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), while different letters indicate significant difference (P<0.05).

Discussion

3.1 Effects of Long-Term Starvation on Juvenile Gibel Carp Animal growth is directly related to food intake. Different species and life stages exhibit varying starvation tolerance. In fish, starvation directly affects larval survival, with the irreversible starvation point for newly hatched larvae being 6-10 days. Juveniles can tolerate months of starvation, but prolonged starvation leads to growth arrest or even negative growth. Starvation significantly impacts reproductive performance in adults, causing abnormal or arrested gonadal development. After short-term starvation and re-feeding, fish body shape, digestive tract structure and function can gradually return to normal, with compensatory growth sometimes occurring. However, the effects of long-term starvation are irreversible, requiring longer time to restore normal body shape and resulting in persistent growth lag. In this study, juvenile gibel carp showed significant decreases in body weight and condition factor after 90 days of starvation. Although feeding and growth resumed after re-feeding, growth was clearly delayed.

Starvation-induced consumption of energy reserves typically manifests as significantly reduced abdominal fat, disintegrated liver tissue, atrophied hepatocytes, and emaciation. Body composition changes include decreased protein and lipid content in body and viscera tissues. Long-term starvation also alters digestive tract morphology, causing cell reduction and atrophy, loose muscle fiber arrangement, and intestinal villi damage. In this study, long-term starvation caused weight loss and atrophy of liver and intestine in juvenile gibel carp. Digestive enzyme activities initially increased then gradually decreased, stabilizing at low

levels from day 60, indicating that starvation exceeding 60 days had stressed the fish. The consumption process during starvation generally utilizes fat tissue first, followed by protein. This principle is applied in aquaculture to reduce fish body fat content, though short-term starvation is ineffective. Xia et al. found that 15 days of starvation could not significantly reduce grass carp fat content, while 50 days caused significant decrease. Although continuous monitoring of body composition during starvation was not conducted in this study, the significant decreases in both crude protein and crude lipid content after 90 days of starvation indicated that long-term starvation reduces both fat and protein levels.

3.2 Protein Requirement of Starved Juvenile Gibel Carp Extensive research has been conducted on gibel carp nutritional requirements, showing that different protein levels affect growth, digestive physiology, and immune metabolism. Optimal dietary protein levels vary significantly among life stages: 40%-45% for larvae and 30%-40% for juveniles. High-protein diets promote gonadal development and fecundity. Even at similar growth stages, protein requirements may differ due to diet formulation and culture environment. Zhao et al. reported that high feeding frequency and high protein level increased feed intake, SGR, and feed efficiency in gibel carp, with growth rate directly related to feed intake. When dietary protein level is low, feeding frequency can be increased to meet nutritional requirements. Under certain conditions, low-protein diets may increase feed intake, possibly due to compensation for lower dry matter and digestible energy.

In this study, growth rate increased and FCR decreased with higher dietary protein levels after re-feeding. Considering growth, FCR, and protein sources, the suitable dietary protein level for juvenile gibel carp after long-term starvation was 35%-40%, though the effects of long-term starvation could not be fully compensated by high-protein diets. A higher SGR:FCR ratio indicates better feeding effects and optimal strategies. The P35 and P40 groups showed higher and similar SGR:FCR ratios. A cubic regression equation between dietary protein level (x) and SGR:FCR (y) was established: $y = -0.000276x^3 + 0.02448x^2 - 0.664x + 5.852$, showing that the theoretical maximum SGR:FCR was achieved at 38.1% dietary protein, suggesting the theoretical optimal protein level is 38.1%. The quadratic regression-derived theoretical optimal level was higher than the actual optimal value, indicating that both the actual optimal value and theoretical level may be suitable for recovery growth, with little variation in SGR and FCR within this range.

3.3 Adaptation Process of Starved and Re-fed Juvenile Gibel Carp Fish starvation tolerance is closely related to environmental conditions, particularly temperature, which directly affects feeding. Within the suitable temperature range, feed intake is positively correlated with temperature. Fish continue feeding during overwintering and may gain weight. Generally, warm-water fish reduce or cease feeding when water temperature drops below 5 °C. Studies

show that stopping feeding before winter improves overwintering survival, as satiation feeding at low temperatures affects physiological function more than starvation. In aquaculture, feeding strategies are adjusted according to fish feeding habits, with reduced feeding frequency and amount as water temperature decreases. At low temperatures, metabolic rate decreases, nutrient utilization efficiency declines, and energy allocation mechanisms change to maximize weight preservation. After temperature recovery, basal metabolism increases, activity enhances, and fish gradually resume feeding and growth.

In this study, the 15 °C temperature during starvation resulted in low metabolic rate and activity, slow material and energy consumption, but long-term starvation still compromised digestive and metabolic functions, affecting nutrient utilization after re-feeding. The degree of nutritional stress determines the compensation strategy, achieved primarily through increased feed intake and feed conversion efficiency.

Generally, SGR is higher in early developmental stages than later stages. In this study, SGR was low and FCR was high during early re-feeding, indicating that most consumed energy was used for physiological and metabolic function recovery rather than growth. Later re-feeding stages showed increased SGR and decreased FCR, with more energy allocated to compensatory growth. After 56 days of re-feeding, the P20 and P25 groups showed lower body weight and condition factor with higher FCR, indicating these diets were unsuitable for starved juveniles. However, during the initial re-feeding phase (days 1-14), dietary protein level had no significant effect on growth, and FCR was high across all groups. No significant differences in body weight were observed, and low-protein groups showed higher feeding activity than high-protein groups. Regression analysis of SGR and FCR also indicated lower protein requirements during early re-feeding than later stages.

This may be because starvation stress severely affected feeding and digestive functions. During initial re-feeding, fish primarily adapted to the feed, with differential adaptation to protein levels related to organ structure and function. After long-term starvation, liver and digestive tract atrophy resulted in low digestive enzyme activity. The degenerated digestive function may have adapted better to low-protein diets, allowing fish to obtain more material and energy through increased feed intake. High-protein diets may have imposed greater metabolic stress and been difficult to adapt to and utilize quickly. As feeding normalized and digestive organ structure and function recovered, the capacity to utilize high-protein diets improved, with feeding and metabolic levels gradually increasing and growth accelerating. In this study, digestive enzyme activities were higher in later than earlier re-feeding stages, with protease activity higher in high-protein groups. Changes in intestinal tissue morphology and digestive enzyme activities reflected adaptation status to different protein levels. Excessive protein levels during initial re-feeding may cause metabolic stress and resource waste. Using lower-protein diets to adapt feeding and restore digestive function before providing higher-protein diets may be more conducive to

growth recovery. However, further research is needed on material and energy metabolism during early re-feeding to determine actual nutritional requirements, recovery time, and their relationship for maximizing compensatory growth.

To maintain equal energy levels among groups, this experiment added different carbohydrate levels within the utilizable range for juvenile gibel carp. Therefore, the effects of different protein levels on growth, physiology, and digestion, as well as adaptation to re-feeding, may have been partially influenced by dietary carbohydrates. Additionally, starved fish with reduced fat content and low metabolic rates may have different requirements and utilization of dietary lipids, energy, and other nutrients compared to normal fish, requiring further investigation. Studying actual nutrient requirements, adaptation processes, and feeding strategies for long-term starved fish can provide insights for recovery, compensatory growth, feed utilization, and water quality management in aquaculture.

Conclusion

1. Juvenile gibel carp exhibit differential utilization and adaptation to varying dietary protein levels after long-term starvation.
2. Dietary protein level has limited impact on growth during initial re-feeding; however, higher protein levels (35%-40%) effectively promote growth after feeding and digestive functions are restored.
3. Regression analysis identified the theoretical optimal dietary protein level as 38.1%.

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Note: Figure translations are in progress. See original paper for figures.