RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 3: 406-411 (March 2024)

Effects of Varying Quantities of Dietary Protein and Lipid on Growth Performance, Blood Biochemical Parameters, Digestive Histology and Enzyme Activity in Juvenile Gold Fish (*Carassius auratus*)

Satyajit Belsare[1], Sandeep Gore[1], Rajiv Rathod[1], Havgiappa Mogalekar[2], Gajanan Kate[1] 10.18805/IJAR.B-4924

ABSTRACT

Background: Goldfishes are used as an ideal model for nutritional studies in larval and juvenile cyprinids. However, of the limited published data that is available on goldfish, protein requirements have varied from 29% to 53% for goldfish larvae. So the present study was undertaken to determine growth performance of goldfish, *C. auratus* fed with different dietary protein and lipid levels to develop nutritionally balanced diets.

Method: In this study, *Carassius auratus* juveniles were fed with six diets containing two protein levels (300 and 400 g/kg) and three lipid levels (40, 80 and 120 g/kg) to evaluate the optimal dietary protein and lipid levels to determine growth performance, blood biochemical parameters, digestive enzyme activity and intestinal and liver histology.

Result: According to the findings, gold fish juveniles when fed a casein-dextrin based semi-purified diet containing 400 g/kg dietary protein and 80 g/kg dietary lipid content, maximum development and feed utilisation are seen. Second-order polynomial regression also suggests that a diet with 400 g/kg protein and 80 g/kg lipid is best for juvenile goldfish growth and feed utilisation.

Key words: Dietary protein, Digestive enzyme, Goldfish, Histological changes.

INTRODUCTION

For successful aquaculture activity diets containing adequate levels of dietary energy as well as appropriate balance of nutrients is necessary to maintain efficient growth and health of animal. From an economic stand point, it is important that protein be utilized for the synthesis of muscle tissue and not for metabolic energy. The alternative energy sources that can be included in thediet to meet the energy needs are carbohydrate and lipid (De Silva and Anderson, 1995). Inclusion of lipids at higher rates to increase dietary energy level is currently the trend in fish feed production. Clearly, it is important to determine the proper levels of incorporation of nutrients in diets to optimize dietary formulations particularly for juvenile.

Goldfish (*Carassius auratus*) is among the most popular aquarium fish which has a high market value in the ornamental fish trade (Lee and Newman, 1997). They are hardy as well as good laboratory species which gives them scientific value for genetic and physiological research (Stickney, 2000) and they could be used as an ideal model for nutritional studies in larval and juvenile cyprinids (Bandyopadhyay *et al*., 2005).

However, of the limited published data that is available on goldfish, protein requirements have varied from 29% dietary proteinfor growing omnivorous goldfish (Lochmann and Phillips, 1994) to 53% for goldfish larvae (Fiogbé and Kestemont, 1995). To date very limited information is available on growth performance of goldfish, *C. auratus* fed with different dietary protein and lipid levels to develop nutritionally balanced diets.

[1]College of Fishery Science, Maharashtra Animal and Fishery Science University, Nagpur-440 006, Maharashtra, India.
[2]College of Fisheries, Dholi-843 105, Bihar, India.

Corresponding Author: Satyajit Belsare, College of Fishery Science, Maharashtra Animal and Fishery Science University, Nagpur-440 006, Maharashtra, India. Email: satyajitbelsare@mafsu.in

How to cite this article: Belsare, S., Gore, S., Rathod, R., Mogalekar, H. and Kate, G. (2024). Effects of Varying Quantities of Dietary Protein and Lipid on Growth Performance, Blood Biochemical Parameters, Digestive Histology and Enzyme Activity in Juvenile Gold Fish (*Carassius auratus*). Indian Journal of Animal Research. 58(3): 406-411. doi: 10.18805/IJAR.B-4924.

Submitted: 02-05-2022 **Accepted:** 06-12-2022 **Online:** 16-12-2022

MATERIALS AND METHODS

Experimental diets

Six semi-purified experimental diets were formulated to contain two levels of protein (300 and 400 g/kg crude protein) and three levels of lipid (40, 80 and 120 g/kg crude lipid). Fish meal and defatted casein were used as dietary protein sources, cod liver oil and soybean oil as lipid source (1:1 w/w) and dextrin was used as source of carbohydrate. Cellulose was used as filler while carboxy methyl cellulose (CMC) was used as pellet binder. Cod liver oil and soybean oil were incorporated in equal proportions into the experimental diets to ensure adequate supply of fatty acids of both n-6 and n-3 series. Different levels of crude protein and lipid were obtained by changing the level of casein (for protein) and

varying the levels of mixture of cod liver oil and soybean oil (for lipid). The level of dextrin was varied to maintain the energy level in the diets.

All the dry ingredients were finely ground and sieved through 40 number mesh size, weighed individually and thoroughly mixed. Cod liver oil and soybean oil (1:1 w/w) were then added to the dry ingredients and mixed again and 300 g/kg water was added to produce dough. The dough was passed through a laboratory pelletizer with a 1 mm diameter die. The resultant wet pellets were dried in oven at 60°C for 8 h. Ingredients and proximate composition of the experimental diets are shown in Table 1.

Experimental procedures

Young ones of Goldfish, *C. auratus* were transported to the aquaculture research laboratory of College of Fishery Science, Nagpur, Maharashtra, India and acclimated to the laboratory condition for one week. The experiment was conducted for a period of eight weeks. Fish with average weight, 1.66±0.018 g (mean±S.D., n=18) were randomly allotted to 18 glass aquaria (0.6×0.3×0.45 m, water depth 40 cm) with 15 fish to each aquarium. A 2×3 factorial design with 3 replicates was used. Three tanks were randomly assigned to each diet. The fish were hand-fed to apparent satiation twice daily (09:00 am and 04:00 pm) and feed intake was recorded. Temperature was maintained at 30±1°C by using thermostatically controlled heaters.

Sample collection and analyses

Fish were batch weighed at every 07 day interval to know the growth status of the fish and mortalities if any, were recorded. At the end of feeding trial, all fish were fasted for 24 h before harvest. Total number and weight of fish in each tank were measured.

Enzyme analysis

At the termination of the experiment three fishes per replicate (n=9 for each treatment) were dissected 24 h after the feeding and the intestine and liver were removed. Protease activity was determined by casein digestion method of Kunitz (1947). Lipase activity was determined based on Cherry and Crandell (1932). α-amylase activity was assayed with 2% (w/v) starch solution as substrate (Rick and Stegbauer, 1974).

Blood plasma assay

Three fishes per replicate (n=9 for each treatment) were used for estimation of plasma glucose, triglycerides (TG) and cholesterol. Each fish was anesthetized using clove oil @ 50 µl per litre of water before taking blood from fish. Blood was drawn from Vena caudal by using a needle (26 gauge) of a sterile disposable syringe (1 ml capacity). Blood was immediately transferred into eppendorf tube containing 2.7% Ethylenediamine tetra acetic acid (EDTA) solution and mixed well in order to avoid clotting of blood. The plasma was separated from blood by centrifuging at 3500 g for 5 min at 4°C. Later the collected plasma was divided into separate aliquots for determining the plasma concentration of glucose, TG and cholesterol using semi-automatic biochemical analyzer (Erba Chem - 5 X).

Histological analysis

Histological analysis was performed to investigate differences in fish liver and intestine as a result of feeding the different experimental diets. At the end of each experiment three fishes per replicate (n=9 for each treatment) were sacrificed and liver and intestine were dissected. The method of Drury and Wallington (1980) was adopted.

Calculations and statistical analyses

The following parameters were calculated:

$$\text{Weight gain (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Ln}W_2 - \text{Ln}W_1}{T} \times 100$$

Where,
W_1= Mean initial body weight of fish (g).
W_2= Mean final body weight of fish (g).
T= Days.

$$\text{Food conversion ratio (FCR)} = \frac{\text{Total feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$$

Experimental data were analysed by one-way and/or two way analysis of variance (ANOVA) using SPSS programme version 16.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for windows to test the significance and correlation analysis where appropriate. When significant difference occurred (P<0.05), the means were compared by Duncan's multiple range test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

During this trial, growth performance and feed utilization was significantly affected by dietary protein (P<0.05) and lipid level (P<0.05). Fish fed with a diet containing 400 g/kg protein with 80 g/kg lipid (17.86 MJ/g) recorded highest weight gain and SGR (P<0.05) (Table 2). Thus, there was a trend of increasing growth performance and feed utilization with increasing inclusion level of dietary energy at each protein level on the basis of WG, SGR, FI, FCR and PER.

There was positive correlation between dietary energy levels and percentage weight gain (r = 0.70, P<0.05). Thus, dietary energy may be the primary source of variation in growth rate with lower weight gain for low energy diets. Hence, fish presumably catabolised dietary protein to meet energy requirements rather than for growth. A gradual increase in growth with each incremental level of dietary lipid (up to 80 g/kg) strengthens the assumption that with increased energy (up to 17.86 MJ/g) more protein was utilised for tissue building.

The level of 400/80 g/kg (17.86 MJ/g) is therefore deemed suitable for optimal growth of young ones of

goldfish, *C. auratus*. These data suggested that 400 g/kg dietary protein could meet protein requirement of young ones of *C. auratus* under these experimental conditions. The dietary protein level for this fish determined in this study is higher than that reported by Lochmann and Phillips (1994) and bears similarity to the finding from studies conducted by Mohanta and Subramanian (2002).

In the present study, growth performance and feed efficiency decreased with increasing dietary lipid levels from 80 to 120 g/kg at the same protein level and suggesting 80g/kg dietary lipid was optimal for this fish. This trend indicates that the extra lipid was not efficiently used for protein sparing. Diets with higher lipid level produced lower final body weight, WG and SGR. This could be due to lack of essential nutrient such as proteins for normal growth and also due to reduction in food consumption of fish.

In general, FCR tended to decrease either with the increase in dietary protein level at the same lipid level or with the increase in dietary lipid level at the same protein level (Table 2). Further, in case of PER, there was a trend of decreasing PER with increasing level of dietary protein and lipids fed.

In the present study, the protease and lipase activity was found to be higher in intestine as compared to liver

Table 1: Ingredients and proximate composition of diets (g/kg DM).

Ingredients	Diet no. (Protein/lipid)					
	Diet 1 (300/40)	Diet 2 (30/80)	Diet 3 (300/120)	Diet 4 (400/40)	Diet 5 (400/80)	Diet 6 (400/120)
Fish meal[a]	100	100	100	120	120	120
Casein[b]	270	270	270	400	400	400
Cod liver oil[c]	20	40	60	20	40	60
Soyabean oil[d]	20	40	60	20	40	60
Dextrin[b]	300	260	220	150	110	70
Vitamin mixture	30	30	30	30	30	30
Mineral mixture	40	40	40	40	40	40
Carboxy Methyl Cellulose[b]	100	100	100	100	100	100
Cellulose[b]	120	120	120	120	120	120
Proximate composition[e] (g/kg dry weight basis)						
Dry matter	917	919	922	920	921	925
Crude protein	294	297	299	418	404	395
Crude lipid	45	80	112	48	83	124
Crude ash	82.8	83.4	83	86.4	86	87.2
Crude fibre	53	53.4	52	55.3	54.8	55
Nitrogen-free extract[f]	442.2	405.2	376	312.3	293.2	263.8
GE (MJ g^{-1})[g]	16.32	17.14	17.95	17.13	17.86	18.76
DE (MJ g^{-1})[h]	12.83	13.63	14.40	14.00	14.65	15.50

a. Fish meal (Moisture: 49 g/kg, Crude protein: 547 g/kg, Crude lipid: 83 g/kg, Crude ash: 190.7 g/kg, Crude fibre: 13.3 g/kg).

b. Obtained from Hi-media Laboratories, Mumbai, India.

c. Sea cod pure cod liver oil, Universal Medicare Private Limited, Mumbai, India.

d. Sweekar refined soybean oil, Marico industries Limited, Mumbai, India.

e. Values represent means of triplicate samples.

f. Nitrogen-free extract, calculated as NFE = 1000 - (Moisture+Crude protein + Crude lipid + Ash + Crude fibre).

g. Gross energy (GE), calculated based on 23.6, 39.5 and 17.2 KJ g^{-1} for protein, lipid and NFE, respectively.

h. Digestive energy (DE), calculated as per Espinós *et al.,* 2003.

Table 2: Growth and, feed utilization parameters of *C. auratus* fed experimental diets containing various protein and lipid levels.

Diet no.	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Two-way anova		
Protein/lipid	(300/40)	(300/80)	(300/120)	(400/40)	(400/80)	(400/120)	Protein	Lipid	P × L
IBW (g)	1.66±0.02	1.66±0.03	1.66±0.02	1.67±0.02	1.67±0.01	1.67±0.01	-	-	-
FBW (g)	3.09±0.03[a]	3.31±0.03[b]	3.52±0.04[c]	3.62±0.02[d]	3.78±0.02[e]	3.54±0.01[cd]	0.000	0.000	0.000
WG (%)	85.55±1.39[a]	99.52±2.32[b]	111.95±2.67[c]	116.31±1.12[c]	126.07±0.65[d]	112.34±0.43[c]	0.000	0.000	0.000
SGR	1.10±0.01[a]	1.23±0.02[b]	1.34±0.02[c]	1.38±0.01[c]	1.45±0.01[d]	1.34±0.01[c]	0.000	0.000	0.000
FI	2.68±0.03[e]	2.51±0.03[d]	2.37±0.03[c]	2.29±0.01[b]	2.19±0.01[a]	2.23±0.01[ab]	0.000	0.000	0.000
FCR	2.68±0.01[e]	2.27±0.01[d]	1.98±0.03[c]	1.87±0.01[b]	1.70±0.01[a]	1.86±0.01[b]	0.000	0.000	0.000
PER	1.27±0.01[a]	1.48±0.01[c]	1.68±0.03[d]	1.27±0.01[a]	1.45±0.01[c]	1.36±0.01[b]	0.000	0.000	0.000

Values represent mean±SD of three replicates and values within the same row with different letters are significantly different (P<0.05).

Table 3: Protease and lipase activities of intestine and liver in *C. auratus* fed experimental diets for eight weeks.

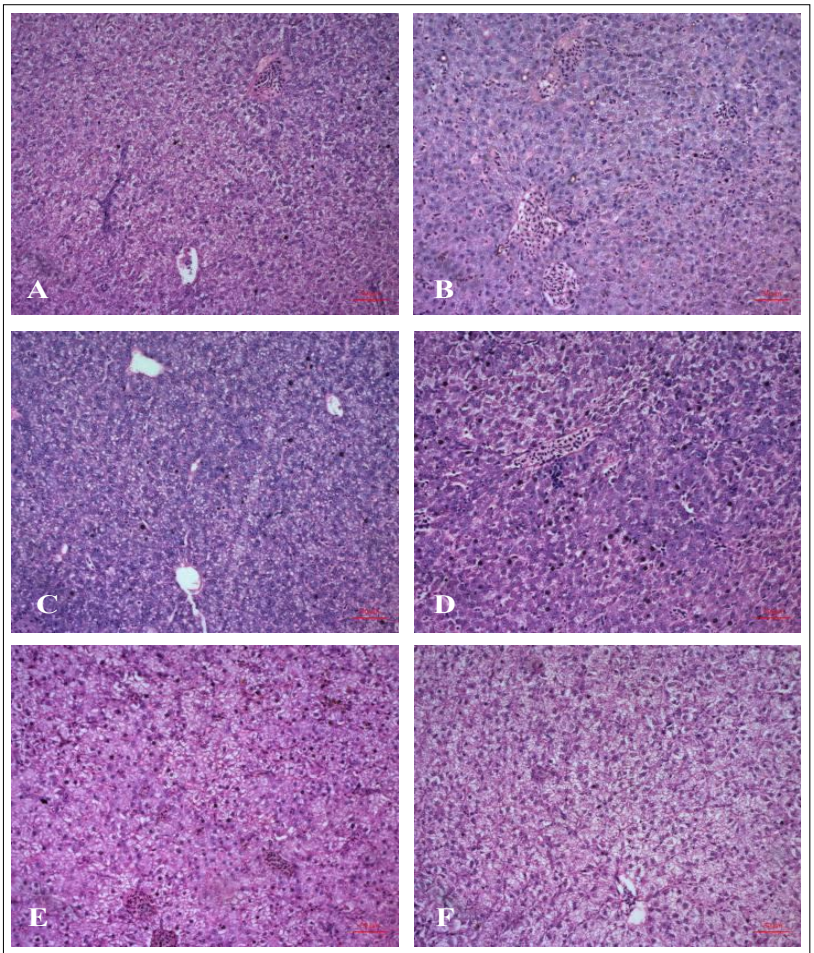
Parameters	Diets (Protein/lipid)						Anova (P-value)		
	T_1 (300/40)	T_2 (300/80)	T_3 (300/120)	T_4 (400/40)	T_5 (400/80)	T_6 (400/120)	Protein	Lipid	Protein × Lipid
Intestinal protease	12.11±0.26^{e}	12.26±0.08^{de}	12.44±0.14^{cd}	12.66±0.12^{b}	13.19±0.12^{a}	12.60±0.09^{bc}	0.000	0.000	0.000
Intestinal lipase	0.76±0.04^{f}	0.86±0.03^{e}	0.91±0.02^{d}	0.98±0.04^{c}	1.21±0.03^{a}	1.06 ±0.03^{b}	0.000	0.000	0.000
Liver protease	9.00±0.02^{c}	9.14±0.04^{b}	9.25±0.10^{b}	8.80±0.03^{d}	9.45±0.13^{a}	9.15±0.05^{b}	0.902	0.000	0.000
Liver lipase	0.59±0.05^{d}	0.67±0.02^{c}	0.73±0.02^{b}	0.78±0.03^{b}	0.95±0.03^{a}	0.91±0.02^{a}	0.000	0.000	0.023

Activities are expressed: protease - micromol of tyrosine released/min/g protein; lipase - units/mg protein. Values represent mean±SD of three replicates and values within the same row with different letters are significantly different (P<0.05).

Table 4: Blood plasma level of glucose, triglyceride and cholesterol of *C. auratus* fed experimental diets for eight weeks.

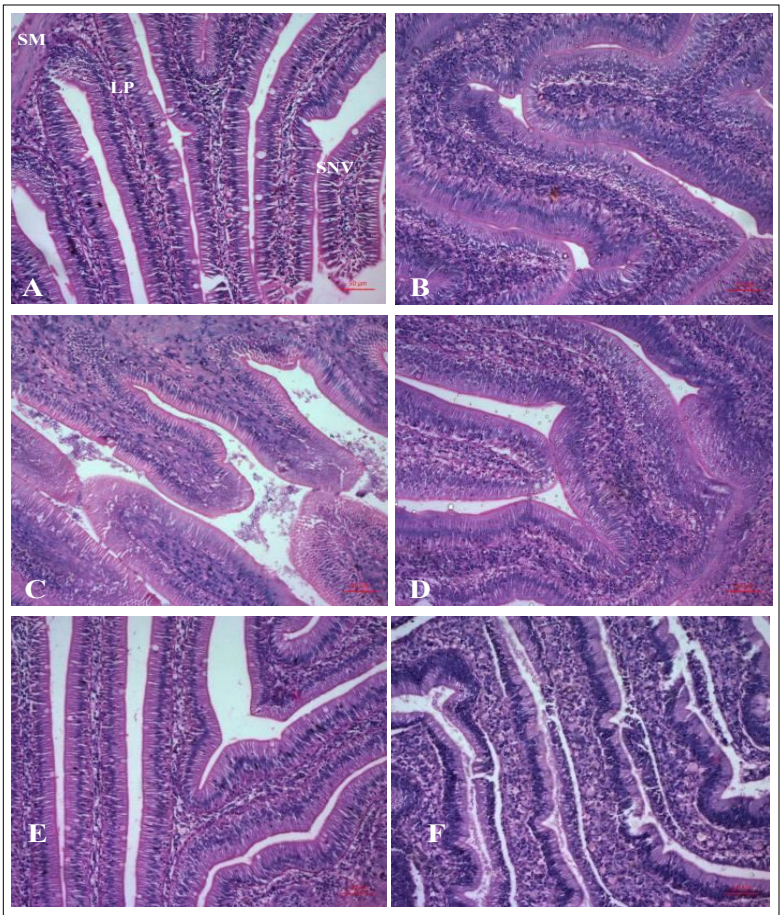
Parameters	Diets (Protein/lipid)						Anova (P-value)		
	T_1 (300/40)	T_2 (300/80)	T_3 (300/120)	T_4 (400/40)	T_5 (400/80)	T_6 (400/120)	Protein	Lipid	Protein × lipid
Glucose (mg/dL)	39.48±0.33^{d}	43.83±0.65^{c}	52.76±2.05^{a}	37.75±0.90^{d}	43.26±1.30^{c}	48.76±1.20^{b}	0.003	0.000	0.079
Triglyceride (mg/dL)	324.63±5.25^{c}	342.33±7.55^{b}	394.37±8.46^{a}	287.67±3.78^{d}	277.70±3.51^{d}	319.70±2.55^{c}	0.000	0.000	0.000
Cholesterol (mg/dL)	118.03±1.59^{f}	137.43±2.20^{c}	161.30±2.23^{a}	126.47±3.35^{e}	132.87±2.91^{d}	149.37±2.19^{b}	0.040	0.000	0.000

Values represent mean±SD of three replicates and values within the same row with different letters are significantly different (P<0.05).



(A) Fish fed 300/40 P/L diet; (B) Fish fed 300/80 P/L diet; (C) Fish fed 300/120 P/L diet; (D) Fish fed 400/40 P/L diet; (E) Fish fed 400/80 P/L diet; (F) Fish fed 400/120 P/L diet. (H-E staining). Scale bar: 50 µm.

Plate 1: Histology of liver (×200) of *C. auratus* fed experimental diets varying in protein to lipid levels (P/L).



(A) Fish fed 300/40 P/L diet; (B) Fish fed 300/80 P/L diet; (C) Fish fed 300/120 P/L diet; (D) Fish fed 400/40 P/L diet; (E) Fish fed 400/80 P/L diet; (F) Fish fed 400/120 P/L diet. Supranuclear vacuole (SNV), lamina propria (LP) and sub-epithelial mucosa (SM). (H-E staining). Scale bar: 50 µm.

Plate 2: Histology of intestine (×200) of *C. auratus* fed experimental diets varying in protein to lipid levels (P/L).

(Table 3). Similar observations have been reported in Mahseer (Bazaz and Keshavnath, 1993) and African catfish (Ali and Jauncey, 2005). Results of the present study are in consensus with Kawai and Ikeda (1972) and Gangadhara *et al*. (1997). The lipase and protease activity of intestine and liver increased with higher levels of dietary lipid fed in the present study and is consistent with the findings of Bazaz and Keshavnath (1993).

In our study, the increased dietary lipid resulted in higher glucose content in plasma. This is consistent with the conclusion of Aminikhoei *et al*. (2015). Cholesterol levels are influenced by the diet of fishes (Regost *et al*., 2001). In the present study, plasma TG and cholesterol level was significantly affected ($P<0.05$) by dietary protein and lipid levels (Table 4). The levels of plasma TG and cholesterol were positively correlated with dietary lipid levels (40, 80 and 120 g kg^{-1}) at both protein levels (300 and 400 g kg^{-1}). The present study indicated active lipid transport due to higher dietary lipid level.

During the present study, no parenchymal cell damage and glycogen deposition in cellular vacuoles of the liver and intestine of *C. auratus* was observed, while some vacuolization was observed in liver of fishes fed with dietary protein and lipid level of 400/80 g kg^{-1} and 400/120 g kg^{-1} (Plate 1 and 2). Lipid droplets in hepatocytes as a physiological response to extra lipid have been observed in various fishes by different authors (Kestemont *et al*., 2001).

CONCLUSION

Present study revealed that growth performance, digestive enzymes and blood plasma components of young ones of goldfish, *C. auratus* are influenced by dietary protein and lipid levels. Maximum growth and better feed utilization was recorded when fed a casein-dextrin based semi-purified diet containing 400 g kg^{-1} dietary protein and 80 g kg^{-1} dietary lipid level. Protein sparing action was observed by increasing dietary lipid levels from 40 g kg^{-1} to 80 g kg^{-1}. This shows that lipid requirement of *C. auratus* juveniles is 80 g/kg, which was further analysed by the second-order polynomial regression for optimization. From regression analysis ($y=1.123+0.0871x - 0.0057x^2$, $R^2=0.983$) for 400 g/kg dietary protein, it was found that the optimum lipid level of this species is 80 g/kg diet.

Conflict of interest

All authors declared that they have no conflict of interest.

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