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Research Article

Effect of replacing fishmeal with cricket meal on growth performance, muscle composition, serum biochemical indices, and antioxidant capacity in juvenile *Pelteobagrus fulvidraco*

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Abstract

Five isonitrogenous and isoenergetic experimental diets of juvenile *Pelteobagrus fulvidraco* were formulated by replacing 0%, 15%, 30%, 45%, and 60% of fishmeal with cricket meal, named T0, T15, T30, T45, and T60, respectively. The experimental fish were cultured for 10 weeks. The results showed that with an increase in cricket meal content, the final body weight, weight gain rate, and specific growth rate of juvenile *P. fulvidraco* increased first and then decreased. The FBW, WGR, and SGR in T30 were significantly higher than those of T0, whereas the feed conversion ratio was significantly lower than those of T0 and T15. The hepatosomatic index in T30 was significantly higher than those of T0 and T15. The essential amino acid contents in juvenile *P. fulvidraco* muscle, arginine, and valine in T60 were significantly higher than those of T0. Compared with the T0, the content of glucose in the serum of T30, T45, and T60 had significantly increased, whereas the content of total cholesterol was significantly decreased. The activities of the serum superoxide dismutase and catalase in T30 and T60 were significantly higher than that of T0. In conclusion, these results suggested that replacing fishmeal with cricket meal has no effect on growth performance and muscle amino acid contents, and improves serum antioxidant capacity in juvenile *P. fulvidraco*.

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Introduction

Fishmeal has long been considered an important protein source in aquatic feeds because of its rich, balanced amino acid content, good palatability, rich vitamins and minerals, and ease to be digested and absorption (Homska *et al*., 2022). In recent years, on the one hand, with the increase of fish farming and the expansion of farming scale, the demand for fishmeal has increased; on the other hand, the long-term excessive fishing, marine environmental pollution and international supervision and protection of fisheries resources have reduced the number of wild fish caught (Parolini *et al*., 2020). It has caused the price of fishmeal to soar in recent years, which not only increases the feed cost of farmers, but also greatly limits the use of fishmeal in aquafeeds. All of those have a great influence on the sustainable development of aquaculture. Therefore, it is urgent to explore economic, safe, and widely available and environmentally friendly protein sources for replacing fishmeal in aquaculture (Kim, 2022; Mikolajczak *et al*., 2022).

Crickets belong to the Orthoptera order of insects, which contains over 1400 species in the world and more than 30 species have been named, and were distributed throughout China (Zeng *et al*., 2013). Crickets are omnivorous, display great environmental adaptability and fast propagation. Studies have shown that cricket meal has 58%-78% crude protein content, which is much higher than other insects, and rich contains vitamin B_{12}, iron, calcium, and high unsaturated fatty acids (Yoon *et al*., 2019; Murugu *et al*., 2021), which is considered to be one of the new insect protein sources. Taufek *et al.* (2016) showed that replacing 75% and 100% of fishmeal with cricket meal in diets (35% crude protein) improved the growth performance of juvenile African catfish (*Clarias gariepinus*). More recently, a report by Jeong *et al*. (2021) found that replacing not more than 20% of fishmeal with cricket meal has no adverse impact on growth performance in olive flounder (Jeong *et al*., 2021). These results suggested that cricket meal can be used as a protein source to replace fishmeal in aquaculture. In addition, a study has shown that cricket meal can also be used as a high protein source in pet diets and that 24% of the supplemental level has no adverse effect on the growth of adult pet dogs (Kilburn *et al*., 2020).

Pelteobagrus fulvidraco is a kind of broad-temperature freshwater economical fish widely distributed in the rivers of China. Because of its tender flesh, delicious taste, no intermuscular spines, and high nutritional value, it is loved by consumers and has become one of the major cultured fish (Su *et al*., 2017). *P. fulvidraco* is an omnivorous animalistic fish, and the demand for fishmeal in the diets of juvenile fish is high, accounting for more than 35% (Qin *et al*., 2019). At present, there is no report on the application of cricket meal as a substitute for fishmeal in the diets of juvenile *P. fulvidraco*. Therefore, this study investigated the growth performance, muscle composition, serum biochemical indexes, and antioxidant capacity of juvenile *P. fulvidraco* by replacing different proportions of fishmeal in the diets with cricket meal. Our aim is to explore the feasibility of replacing fishmeal in diets of

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P. fulvidraco and provide a scientific reference for the development and application of insect protein sources in aquatic feeds in the future.

Materials and methods

Experimental fish

Juvenile *P. fulvidraco* (2.0±0.13 g) was purchased from a freshwater fish farm located in Jili city (Luoyang, China). The 450 healthy fish were selected and disinfected with 3% germicidal brine and put into the temporary pond.

Experimental diet formulation

The crickets (*Gryllus bimaculatus*) were purchased from the wholesale market of Chinese herbal medicine in Bozhou, Anhui Province, China, dried and crushed, and then stored at low temperatures. The cricket meal was formulated to contain 63.40% crude protein, 15.50% crude lipid and 7.36% crude ash, respectively. Fishmeal, blood meal, and soybean meal were used as the main protein sources, fish oil, and soybean oil as the main lipid sources to prepare the basic diet. On this basis, cricket meal was used to replace 0%, 15%, 30%, 45% and 60% of fishmeal in the basal diet to formulate five groups of isonitrogenous (44.0% crude protein content) and isoenergetic (19.00 MJ/kg) experimental diets, named in order as T0 (0% replacement), T15 (15% replacement), T30 (30% replacement), T45 (45% substitution) and T60 (60% substitution). The formula and nutritional composition of the experimental diet are presented in Table 1, and the amino acid (AA) contents of fishmeal, cricket meal and the experimental diets of each group are presented in Table 2.

Table 1: Composition and nutrient levels of experimental diets (% in dry matter).

Items	Diet groups				
	T0	T15	T30	T45	T60
Ingredients					
Fishmeal[1]	40.00	34.00	28.00	22.00	16.00
Cricket meal	0.00	6.00	12.00	18.00	24.00
Soybean meal	10.00	10.00	10.00	10.00	10.00
Corn gluten meal	10.00	10.00	10.00	10.00	10.00
Blood meal	4.50	4.50	4.50	4.50	4.50
Wheat flour	25.90	25.90	25.90	25.90	25.90
Mixed oils[2]	5.00	5.00	5.00	5.00	5.00
Squid ointment	1.00	1.00	1.00	1.00	1.00
Premix[3]	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.50	0.50	0.50	0.50	0.50
$Ca(H_2PO4)_2$	1.10	1.10	1.10	1.00	1.00
CMC[4]	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Nutrient levels[5]					
Moisture	9.45	9.50	9.51	9.64	9.75
Crude protein	44.28	44.29	44.17	43.09	44.03
Crude lipid	12.31	12.25	12.21	12.18	12.15
Gross energy (kJ/g)	19.43	19.39	19.28	19.17	19.15

Notes: 1. Fishmeal (65.03% crude protein and 13.46% crude lipid) was provided by Qingdao SurGreen Biological Engineering Co., Ltd. 2 mixture of soybean oil and fish oil; 3. The premix provides minerals and vitamins for a kilogram of diets: K 30.0 mg, Ca 300.0 mg, Mg 10.0 mg, Zn 3.0 mg, Fe 22.0 mg, Cu 1.7 mg, I 0.29 mg, Se 0.02 mg, V_A 8000 IU, V_C 230 mg, V_D 800 IU, V_E 90 mg, V_K 8mg, VB_1 10 mg, nicotinic acid 100 mg, pantothenate acid 50mg, VB_6 15 mg, VB_{12} 10 mg, inositol 150 mg; 4. Sodium Carboxymethyl Cellulose, CMC; 5. The feed nutrition level was measured in values. T0: 0% fishmeal replacement group; T15: 15% fishmeal replacement group; T30: 30% fishmeal replacement group; T45: 45% fishmeal replacement group; T60: 60% fishmeal replacement group.

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Table 2: AA composition of fishmeal, cricket meal and the experimental diets (as is, % dry matter).

Items	Fishmeal	Cricket meal	Diet groups				
			T0	T15	T30	T45	T60
Essential amino acid, EAA							
Arginine	2.58	4.37	1.20	1.29	1.29	1.29	1.33
Histidine	1.62	1.30	0.42	0.41	0.38	0.36	0.35
Isoleucine	2.75	2.80	0.9	0.9	0.91	0.93	0.93
Leucine	4.64	5.19	1.54	1.56	1.27	1.58	1.64
Lysine	4.75	3.27	1.71	1.68	1.66	1.66	1.60
Methionine	1.82	1.10	0.44	0.43	0.42	0.35	0.33
Phenylalanine	2.69	1.96	0.8	0.73	0.7	0.68	0.66
Threonine	1.60	1.55	0.43	0.42	0.42	0.4	0.4
Valine	3.39	4.97	1.24	1.26	1.34	1.38	1.39
Non-essential amino acid, NEAA							
Alanine	4.20	5.84	1.15	1.17	1.22	1.26	1.31
Aspartic acid	5.60	5.69	2.33	2.33	2.33	2.34	2.35
Glycine	4.05	3.31	0.75	0.72	0.70	0.68	0.68
Glutamic acid	8.46	7.23	3.14	3.12	3.09	3.04	2.99
Serine	1.78	1.31	0.36	0.34	0.33	0.32	0.32
Proline	2.65	3.03	0.71	0.72	0.72	0.74	0.73
Cystine	0.60	0.19	0.04	0.04	0.03	0.02	0.02
Total amino acid	23.18	53.11	17.16	17.12	17.11	17.03	17.03

Notes: T0: 0% fishmeal replacement group; T15: 15% fishmeal replacement group; T30: 30% fishmeal replacement group; T45: 45% fishmeal replacement group; T60: 60% fishmeal replacement group.

The ingredients were crushed and sieved at 80-mesh, mixed with fishmeal and cricket meal proportionally, added certain distilled water, and then made into pellet feed with a particle size of 2.5 mm by QRLS-400 electric meat grinder. After air drying at an ambient temperature of 26-30℃, then put them into sealed vacuum-packed plastic bags and store them in the refrigerator at 4℃ before use.

Experimental design and feeding management

The experiment was carried out in a recirculating aquaculture system at Henan University of Science and Technology Aquaculture Laboratory. The water source was tap water and well-aerated before use. Juvenile *P. fulvidraco* were transported to the Experimental Center of Animal Sciences in the Kaiyuan campus of Henan University of Science and Technology (Luoyang, China). The fish were fed with basic diet (Guangdong Yuehai feeds) in outdoor polyethylene tanks each containing 4000 L aerated tap water for four weeks. Thereafter, 450 healthy fish were selected and released into an indoor recirculating aquaculture system. The recirculating aquaculture system comprises 15 glass fiber tanks (diameter 80 cm, height 70 cm) each containing 250 L aerated tap water.

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The fish were acclimated in the experimental tanks for one week, and then were selected and randomly distributed into five groups, with three replicates of 30 fish per tank, and the initial body weight and length were measured. All groups were fed until the juveniles were satiated, twice a day (8:00 am and 17:30 pm). The water temperature was kept at 24.0~30.0 ℃, pH 7.8~8.0, dissolved oxygen >5.0 mg/L, ammonia <0.05 mg/L and nitrite <0.1 mg/L during the experiment period.

Sample collection

At the end of the feeding experiment, all fish were fasted for 24 h, then anesthetized with eugenol (1:10,000). The fish were collected from each tank for measurement of body weight to calculate growth performance. Six fish were randomly selected from each tank for blood sampling by puncture of the caudal vein for biochemical parameter analyses. Blood was collected with a 1.0 mL syringe and transferred into a 1.5 mL Eppendorf tube. The serum was obtained after centrifugation (4,000×g for 10 min at 4°C) and immediately stored at -80 ℃ until analysis.The muscle was taken from the epaxial muscle of fish and stored at -80 ℃ for the determination of amino acid composition.

Proximate composition and amino acids composition analysis

Crude protein and crude lipid of diets were determined by the Kjeldahl method (Kjeltec 2100 Distillation Unit, Foss Tecator, Hoganas, Sweden) and the Soxhlet petroleum-ether extraction method (Soxtec TM 2043 Fat Extraction System, Foss Tecator, Sweden), respectively. Moisture content was determined by drying in an oven at 105 ℃. Gross energy was measured by an oxygen bomb calorimeter (ZDHW-8000, China). All amino acids (AA) contents were detected by an automatic amino acid analyzer (Model Hitachi L-8900, Hitachi, Ltd., Tokyo, Japan) after hydrolyzing the samples with 6 M hydrochloric acid (HCl) at 110℃ for 24 h.

Growth performance and calculation formula

Survival rate (SR, %) =100×(final number of fish)/(initial number of fish);
Weight gain rate (WGR, %) =100×(final body weight-initial body weight)/initial body weight; Specific growth rate (SGR,%/d) =100×[ln final body weight (g)-ln initial body weight (g)]/days; Feed conversion ratio (FCR) =amount of feed given (g)/weight gain (g);
Condition factor (CF) =body weight (g)×100/body length $(cm)^3$;
FI (g/day/individual fish) = (total dry feed intake)/(days × the number of fish)
Viscerosomatic index (VSI, %) =100×visceral weight (g)/body weight (g); and,
Hepatosomatic index (HSI, %) =100×hepatopancreas weight (g)/body weight (g)

Serum biochemical indexes and antioxidant capacity determination

The serum biochemical indexes were determined by the Hitachi 7100 automatic biochemical analyzer. The contents of

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alanine aminotransferase (ALT), aspartate transaminase (AST), total protein (TP), urea nitrogen (UN), glucose (GLU), triglyceride (TG), total cholesterol (TCHO) were measured spectrophotometrically with an automated analyzer (SPOTCHEM™ EZ model SP4430, Arkray, Inc., Kyoto, Japan) at the New District Hospital Laboratory Center of the First Affiliated Hospital of Henan University of Science and Technology. Antioxidant index, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-P_X), the activities were determined by using the kit produced by Nanjing Jiancheng Bioengineering Institute according to the instructions.

Data statistical analysis

Data are presented as mean ± standard error (SE). Statistical analyses were conducted using one-way analysis of variance (ANOVA) and compared with Duncan's multiple range tests. When a normal distribution and/or homogeneity of the variances was not achieved, data were subjected to the Kruskal-Wallis H nonparametric test, followed by the Games-Howell nonparametric multiple comparison test. $p<0.05$ was regarded as the statistically significant level. All statistical analyses were performed using the SPSS 20.0 software.

Results

Growth performance

According to Table 3, there were no significant differences in VSI, FI, CF and SR from T15 to T60 compared to T0 ($p>0.05$). The FBW, WGR, and SGR values in T30 increased significantly compared to T0, while the FCR value was significantly decreased ($p<0.05$); FBW, WGR, and SGR in T15, T45 and T60 did not differ significantly compared to T0 ($p>0.05$). The HSI increased significantly in T30 ($p<0.05$) by comparison between T0 and T15, but not significantly between T45 and T60, but slightly decreased ($p<0.05$).

Table 3: Effects of substituting fishmeal with cricket meal on juvenile *Pelteobagrus fulvidraco* growth performance.

Items	Diet groups				
	T0	T15	T30	T45	T60
FBW (g)	24.30±3.74^{a}	25.79±2.54^{a}	27.06±3.35^{b}	22.34±2.94^{ab}	21.60±1.45^{ab}
WGR (%)	1225.04±69.11^{a}	1284.46±63.07^{a}	1338.65±87.21^{b}	1169.77±79.01^{ab}	1120.85±55.23^{ab}
SGR(%)	4.78±0.11^{a}	4.82±0.09 ^{a}	4.95±0.10 ^{b}	4.47±0.11^{ab}	4.34±0.19^{ab}
FI (g/fish/day)	26.12±0.15	26.47±0.23	27.47±0.20	26.07±0.15	26.01±0.25
VSI (%)	7.71±0.29	7.82±0.27	7.59±0.34	7.84±0.19	7.51±0.20
HIS (%)	1.62±0.21 ^{a}	1.60±0.10^{a}	1.78±0.09^{b}	1.71±0.12^{ab}	1.69±0.18^{ab}
CF/(g/cm^3)	2.02±0.03	2.11±0.04	2.16±0.01	2.09±0.07	2.01±0.04
SR (%)	98±1.01	97±1.02	98±1.34	99±1.21	97±1.25

Notes: In the same row, values with different small letter superscripts show significant differences ($p < 0.05$). FBW: final body weight; WGR: weight gain rate; SGR: specific growth rate; FI: feed intake; VSI: viscerosomatic index; HIS: Hepatosomatic index; CF: condition factor; SR: survival rate. T0: 0% fishmeal replacement group; T15: 15% fishmeal replacement group; T30: 30% fishmeal replacement group; T45: 45% fishmeal replacement group; T60: 60% fishmeal replacement group.

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Muscle AA composition analysis

According to the nutritional composition of cricket meal determined in Table 2, Lys and Met content are slightly lower in EAA than fishmeal; while Arg content is higher than fishmeal. The AA composition of the juvenile *P. fulvidraco* muscles detected nine essential amino acids (EAAs) and eight non-essential amino acids (NEAAs) (Table 4). The replacement of cricket meal with different proportions of fishmeal had no significant effect on the His, Thr, Met, Phe, Ile, Leu, and Lys and TEAA and TNEAA contents in the muscles of juvenile *P. fulvidraco* (p>0.05); while Arg and Val contents were significantly higher in T60 than in T0 and T15 (p<0.05). In NEAA, the Glu content in the replacement groups did not differ significantly from T0 (p>0.05), except that T60 was significantly lower than T0 (p<0.05); none of the other NEAA contents were found to be significantly difference among groups (p>0.05). In addition, the replacement of cricket meal with different proportions of fishmeal had no significant effect on the contents of Asp, Gly, Ala, and total flavor amino acid (TFAA) in the muscle of juvenile *P. fulvidraco* (p>0.05).

Table 4: Effects of the replacement of fishmeal with cricket meal on muscle AA composition in juvenile *Pelteobagrus fulvidraco* (as is, % dry matter).

Amino acids	Diet groups				
	T0	T15	T30	T45	T60
EAA					
His	1.59±0.10	1.55±0.13	1.51±0.14	1.50±0.17	1.48±0.19
Thr	1.47±0.44	1.45±0.06	1.43±0.04	1.41±0.01	1.39±0.36
Arg	1.45±0.65^{a}	1.48±0.03^{a}	1.62±0.02^{ab}	1.61±0.01^{ab}	1.79±0.22^{b}
Val	3.33±0.88^{a}	3.39±0.03^{a}	3.40±0.01^{a}	3.47±0.03^{ab}	3.60±0.35^{b}
Met	1.55±0.01	1.53±0.01	1.52±0.03	1.51±0.02	1.46±0.06
Phe	0.86±0.44	0.88±0.07	0.86±0.08^{a}	0.84±0.11	0.80±0.12
Ile	1.09±0.02	1.09±0.04	1.10±0.03	1.11±0.05	1.06±0.20
Leu	4.68±1.14	4.70±0.04	4.69±0.03	4.71±0.02	4.58±0.32
Lys	2.07±0.05	2.01±0.05	2.01±0.03	1.93±0.07	1.92±0.04
NEAA					
Asp*	4.75±0.90	4.77±0.05	4.73±0.05	4.76±0.02	4.77±0.39
Glu*	7.12±1.61^{a}	7.10±0.07^{a}	7.01±0.07^{ab}	6.83±0.04^{ab}	6.55±0.47^{b}
Ser	1.73±0.36	1.74±0.02	1.71±0.02	1.68±0.01	1.79±0.13
Gly*	2.79±0.03	2.74±0.02	2.71±0.03	2.70±0.03	2.69±0.05
Ala*	3.40±0.02	3.43±0.02	3.45±0.02	3.46±0.01	3.47±0.03
Try	1.57±0.02	1.62±0.01	1.59±0.02	1.59±0.03	1.57±0.05
Cys	0.12±0.02	0.12±0.03	0.15±0.01	0.15±0.01^{a}	0.14±0.01
Pro	2.78±0.08	2.73±0.09	2.81±0.03	2.79±0.05	2.85±0.02
TFAA	18.06±0.17	18.14±0.10	17.90±0.19	17.75±0.18	17.48±0.25
TEAA	18.10±0.21	18.08±0.08	18.16±0.06	18.09±0.07	18.08±0.04
TNEAA	24.26±0.11	24.25±0.13	24.16±0.16	23.96±0.14	23.83±0.15

Notes: ★Denotes flavor amino acid (FAA). In the same row, values with different small letter superscripts show significant differences (p<0.05). His: histidine; Thr: threonine; Arg: arginine; Val: valine; Met: methionine; Phe: phenylalanine; Ile: isoleucine; Leu: leucine; Lys: lysine; Asp: aspartic acid; Glu: glutamic acid; Ser: serine; Gly: glycine; Ala: alanine; Try: tryptophan; Cys: cystine; Pro: proline; EAA: essential amino acid; NEAA: non-essential amino acids; TFAA: total flavor amino acid; TEAA: total essential amino acids; TNEAA: total non-essential amino acids. T0: 0% fishmeal replacement group; T15: 15% fishmeal replacement group; T30: 30% fishmeal replacement group; T45: 45% fishmeal replacement group; T60: 60% fishmeal replacement group.

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Serum biochemical indices analysis

According to Table 5, the ALT, AST, TP, UN and TG contents were not significantly different with the increasing proportion of cricket meal (p>0.05). The GLU content was increased significantly in T30 (p<0.05), but was not significantly different from T15, T45, and T60 (p>0.05). In addition, the TCHO content of T30 was significantly lower than that of T0 (p<0.05), but its content was not significantly different among the experimental groups (p>0.05).

Table 5: Effects of the replacement of fishmeal with cricket meal on the serum biochemical indices of juvenile *Pelteobagrus fulvidraco*.

Items	Diet groups				
	T0	T15	T30	T45	T60
ALT/(U/L)	11.19±1.12	10.24±2.11	9.89±1.72	9.74±0.78	9.32±0.25
AST/(U/L)	97.21±3.05	96.03±4.61	95.44±6.05	94.47±8.43	90.05±9.14
TP/(g/L)	17.23±1.15	17.11±1.97	19.35±2.01	16.18±2.13	17.43±1.95
UN/(mmol/L)	1.43±0.40	1.40±0.72	1.40±0.08	1.37±0.52	1.32±0.92
GLU/(mmol/L)	3.34±0.81^{a}	3.94±0.21^{ab}	4.53±0.52^{b}	4.41±0.76^{b}	4.37±0.59^{b}
TG/(mmol/L)	7.97±0.40	7.56±0.39	7.44±0.34	7.41±0.29	7.33±0.67
TCHO /(mmol/L)	5.02±0.62^{b}	4.75±0.45^{ab}	3.78±0.72^{a}	3.79±0.78^{a}	3.82±0.72^{a}

Notes: In the same row, values with different small letter superscripts show significant differences (p<0.05). ALT: alanine aminotransferase; AST: aspartate transaminase; TP: total protein; UN: urea nitrogen; GLU: glucose; TG: triglyceride; TCHO: total cholesterol; T0: 0% fishmeal replacement group; T15: 15% fishmeal replacement group; T30: 30% fishmeal replacement group; T45: 45% fishmeal replacement group; T60: 60% fishmeal replacement group.

Serum antioxidant indices analysis

As shown in Table 6, the activities of T-AOC and GSH-Px, and MAD content were not significantly different among all the groups (p>0.05). The replacement of fishmeal with cricket meal in diets could significantly improve the serum SOD activity of juvenile *P. fulvidraco*, and reach the highest in T30 (p<0.05), then followed by a decreasing trend but not significant difference between the T45 and T60. The CAT activity of T0 and T15 were significantly lower than in T30, T45 and T60 with the proportion increasing of cricket meal in diets (p<0.05), and T60 exhibits the highest activity.

Table 6: Effects of the replacement of fishmeal with cricket meal on the serum antioxidant indices of juvenile *Pelteobagrus fulvidraco*.

Items	Diet groups				
	T0	T15	T30	T45	T60
T-AOC (U/mg port)	0.13±0.02	0.15±0.02	0.15±0.03	0.14±0.02	0.11±0.04
SOD (U/mg port)	124.21±26.37a	146.87±17.59ab	169.14±10.03b	152.38±14.36b	139.12±13.31a b
MAD (nmol/mg port)	10.14±1.31	11.31±1.68	9.08 ±2.44	10.13±1.63	9.85±1.14
GSH-Px (U/mg port)	12.11±2.20	11.76±1.86	11.01±1.58	9.34±2.83	12.09±0.73
CAT (U/mg port)	4.85±0.69a	5.03±0.12a	7.89±3.02b	8.41±1.76b	8.61±2.45b

Notes: In the same row, values with different small letter superscripts show significant differences (p<0.05). T-AOC: total antioxidant capacity; SOD: superoxide dismutase; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; CAT: catalase. T0: 0% fishmeal replacement group; T15: 15% fishmeal replacement group; T30: 30% fishmeal replacement group; T45: 45% fishmeal replacement group; T60: 60% fishmeal replacement group.

Discussion

At present, a variety of protein sources as an alternative to fishmeal have been

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reported in the application of *P. fulvidraco* diets, such *Tenebrio molitor* meal, housefly maggot meal, krill meal and *Hermetia illucens* meal (Wen *et al*., 2013; Ji *et al*., 2016; Su *et al*., 2017; Chen *et al*., 2019). However, there are no reports on the replacement of fishmeal by cricket meal in the study of juvenile *Pelteobagrus fulvidraco*. Cricket meal is a kind of insect protein source with a high nutritional value, and has the potential to become a source of functional food and feed protein (Abd El-Wahab *et al*., 2021; Maiyo *et al*., 2022). WGR and SGR are important indicators in the production of cultured fish. At the end of 10 weeks of feeding, FBW, WGR, SGR, FCR, and HSI of juvenile *Pelteobagrus fulvidraco* were significantly increased when 30% of fishmeal in the basal diet was replaced by cricket meal. Whereas, when the replacement ratio was over 45% and 60%, there was a tendency to decrease in FBW, WGR, SGR, FCR and HSI compared with T0, but have no significant impact. The replacement of fishmeal by cricket meal did not affect the SR, indicating that the experimental fish could be well adapted to cricket meal as a substitute for fishmeal. It is worth noting that with the increase of cricket meal replacement level, FBW, WGR and SGR of juvenile *P. fulvidraco* increased at first and then decreased, which may be related to the increase of chitin in cricket meal, affecting the digestion and absorption of feed. The current study also is in line with other studies that illustrated that the fish meal was replaced by cricket meal of 75% and 100% in the diets of African catfish, respectively (Taufek *et al*., 2016). After 7 weeks of feeding, they found that SGR of 75% and 100% of the replacement group was significantly different from that of the control group, and SGR showed a significant increasing trend with the increase of cricket meal replacement, but no significant difference in FCR. There may be three reasons for this result: (1) it is related to the different nutritional value of cricket meal used in this experiment; (2) The nutritional metabolism mechanism of different fish species is different, so the tolerance to cricket meal is also different, leading to the appropriate proportion of cricket meal added in different fish feed; (3) The chitin contained in cricket meal and unknown anti-nutritional factors may alter the feed palatability after replacement affecting the digestion and absorption (Finke *et al*., 1987; Alegbeleye, *et al*., 2012; Psarianos *et al*., 2022). For this phenomenon, the specific mechanism needs to be further studied. Previous research from our experiment shown that when the proportion of cricket meal replacing fishmeal (60% of fishmeal in basic diets) in diets of juvenile *P. fulvidraco* does not exceed 45%, there is no significant reduction in FBW, WGR, SGR and FCR (Wang *et al*., 2022), which also shows that cricket meal replacing fishmeal can affect the growth performance of different kinds of fish. HSI increases gradually with the increase of the proportion of cricket meal replacing fishmeal, and HSI value of T30 is significantly higher than that of T0. Previous studies have shown that the lipid content in feed significantly affects the HSI of fish (Lee *et al*., 2002; Rueda-Jasso *et al*., 2004), which still needs further research.

The quality of animal muscle is closely related to its nutrient content and composition. AA is the basic component of

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protein, and the composition, types and proportion of AA affect the nutritional value of muscle protein. AA content of animal tissues is mainly affected by crude protein and AA content in diets (Jahan *et al*., 2021). There is no significant effect on the contents of most EAA, NEAA, and TFAA in the muscles of juvenile *P. fulvidraco* in the feeding experiment. The contents of Arg and Val in T60 were significantly higher than that of T0. The results indicated that a high proportion of cricket meal instead of fishmeal could affect AA composition in the muscle of juvenile *P. fulvidraco*. It is worth further studying whether the Met content of the first restrictive amino acid is lower in cricket meal than in fishmeal and will affect the AA balance of the body (Han *et al*., 2020). The Glu content in T0 is not significantly different from T15, T30 and T45, but it is significantly higher than T60. The reason for the difference may be that there is a positive correlation between the content of fishmeal and cricket meal in diets. In our present study, the content of Glu in fishmeal and cricket meal is significantly higher than other AA. There was no significant difference in the content of TFAA among all groups, indicating that cricket meal instead of fishmeal had no significant effect on the muscle AA composition of juvenile *P. fulvidraco*. Previous studies confirmed that the lack of Arg, Lys and Met will reduce the growth performance of aquatic animals (Rossi and Davis, 2012), because these AA are involved in many metabolic processes of the body, and are also precursor substances for the synthesis of choline and Cys (Kasper *et al*., 2000; Goff and GatlinIII, 2004). At present, the mechanism of EAAs absorption and metabolism of insect meal in aquatic animals is still unclear.

Serum biochemical indexes can directly reflect the physiological function and nutritional metabolism of fish. In our study, serum TP, UN, ALT, and AST were not significantly altered by dietary treatment. ALT and AST contents in serum can indirectly reflect the liver metabolism. In this study, the contents of ALT and AST in all groups were not significantly altered, indicating that fishmeal was replaced by cricket meal having a negative effect on fish liver or leading to tissue damage. TP is synthesized in the liver, and its content is an important indicator of protein metabolism and nutritional health in the body (Sun *et al*., 2014). Currently, there was no significant alteration in the blood TP concentration of juvenile *P. fulvidraco* among all groups, indicating that the replacement of cricket meal with different proportions of fishmeal had no adverse effect on the protein nutrition and metabolism of fish. GLU content has no significant alteration between T30 and T60, but is significantly higher than T0, and its value reaches the maximum in T30, indicating that the replacement of fishmeal with cricket meal in an appropriate proportion has little effect on sugar metabolism of juvenile *P. fulvidraco*. With the increase of the proportion of cricket meal, TCHO content decreased. Compared with T0, TCHO content in T30, T45, and T60 significantly decreased. This is consistent with other reports that feeding European perch (*Dicentrarchus labrax*) with *Hermetia illucens* meal substituted fishmeal can reduce the cholesterol content

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in plasma (Magalhães *et al*., 2017). The increase of TCHO content in serum mainly comes from the liver, which indicates that the function of liver cells in animals is impaired or damaged, and lipid metabolism is disturbed.

The activities of antioxidant enzymes in the organism are an adaptive protection mechanism for the organism to resist oxidative stress (Helland and Grisdale-Helland, 2006). Studies implied that insect meal can enhance the non-specific immune response of organisms and improve the antioxidant capacity of fish (Sakai *et al*., 1992; Powell and Rowley, 2007; Vahedi and Ghodratizadeh, 2011). Our study showed that with the increase of cricket meal replacement level, the activities of SOD and CAT in the serum of juvenile *P. fulvidraco* increased. SOD activities of T30 and T45 were significantly higher than that of T0, which indicated that cricket meal replacement with fishmeal may cause oxidative stress reaction of juvenile *P. fulvidraco*. However, when the replacement level of cricket meal exceeds 45%, SOD activity shown a decreasing trend. Previous studies have found that feeding diets containing appropriate insects can increase the antioxidant capacity of animals (Maulu *et al*., 2022). It may be related to the excessive cricket meal concentration, which aggravate the physiological metabolism burden of juvenile fish and affect the antioxidant capacity. The total CAT activity in the serum in T30, T45, and T60 were significantly increased compared with that of T0. Liu *et al*. (2017) reported that the replacement of fishmeal with 70% of the *Hermetia illucens* larvae meal could significantly increase the activities of SOD and CAT in the serum of Koi carp, which was consistent with the results of our study. The replacement fishmeal with cricket meal in different proportions had no significant effect on the activities of T-AOC, GSH-Px and MDA content in serum, indicating that there was no adverse effect on the antioxidant immune indexes of juvenile *P. fulvidraco*. MDA is the intermediate product in the process of lipid peroxidation, and its content alteration can be used to evaluate the degree of cell stress damage (Reiser *et al*., 2011; Florescu Gune *et al*., 2021). With the increase of cricket meal replacement level, MDA content in the serum of juvenile *P. fulvidraco* slightly increased, but there was no significant difference among all groups, indicating that cricket meal replacement had no effect on the antioxidant capacity of the serum of juvenile *P. fulvidraco*.

Conclusions

In conclusion, up to 60% replacement of fishmeal with cricket meal in diets of juvenile *P. fulvidraco* exerted no negative impact on the SGR or FCR and does not affect the content of AA in the muscle. Moreover, 30% of FM replacement with cricket meal is beneficial to blood health and can improved the serum antioxidant activities of juvenile *P. fulvidraco*. Therefore, it can be concluded that cricket meal has potential as an ingredient in practical diets.

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Conflicts of Interest

The authors confirm that there is no conflict of interest in this paper.

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