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SPECIAL ISSUE: *Application of insect ingredients in sustainable aquaculture*

Dietary black soldier fly pulp affects growth, antioxidant and immune capacity of *Micropterus salmoides*

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RESEARCH ARTICLE

Abstract

Dried or defatted black soldier fly (*Hermetia illucens*) larvae meal has been shown an important ingredient in aquafeed, but little information is available about the effects of black soldier fly pulp (BSFP) on growth and health of fish. A 62-day feeding trial was conducted to assess the effects of dietary BSFP on growth performance, whole body composition, serum metabolites, antioxidant and immune response of *Micropterus salmoides*. Four isoproteic and isolipidic diets were formulated by adding BSFP (original substance) to the basal diet at the rate of 0% (BSFP0), 1% (BSFP1), 2% (BSFP2) and 4% (BSFP4), corresponding to inclusion of 0, 4.5, 9.0 and 18.0 g/kg DM in diets. Each diet was randomly assigned to triplicate groups of 35 fish per tank. Fish were fed twice daily to apparent satiation. Blood samples were collected at the terminal trial to analyse serum metabolites, antioxidant and immune enzyme activities. Results indicated that fish fed BSFP2 had higher ($P<0.05$) weight gain rate, specific growth rate and intraperitoneal fat ratio than those of fish fed other diets. Crude lipid, ash and calcium contents were higher ($P<0.05$) in BSFP2 than those in BSFP0. Fish fed BSFP2 and BSFP4 had lower ($P<0.05$) serum malonaldehyde but higher ($P<0.05$) acid phosphatase than other diets. It was concluded that dietary inclusion of BSFP improved growth performance of *M. salmoides* might be attributed to increased lipid and ash deposition. BSFP improved antioxidant and immune capacity but also increased intraperitoneal fat deposition of fish, suggesting BSFP should be careful to be used in *M. salmoides* diets.

Keywords: black soldier fly pulp, *Micropterus salmoides*, growth, antioxidant, immune

1. Introduction

In recent years, black soldier fly (*Hermetia illucens* L.) has begun to play an important role in aquaculture as a promising alternative protein source and ingredient in aquafeeds due to its comparable palatability, essential amino acid pattern and nutritive value to fish meal. It has been well reported that black soldier fly is suitable to be used in diets without impairing growth performance of aquatic animals (Cummins *et al.*, 2017; Hu *et al.*, 2017a, 2018, 2019; Lock *et al.*, 2016; Magalhaes *et al.*, 2017; Renna *et al.*, 2017; Sealey *et al.*, 2011; Wang *et al.*, 2019; Xiao *et al.*, 2018; Xu *et al.*, 2020; Zhou *et al.*, 2017). Our previous studies demonstrated that dietary inclusion of black soldier fly larvae meal or black soldier fly larvae oil did not alter growth performance of *Lateolabrax japonicus* (Wang *et al.*, 2019), *Pelteobagrus fulvidraco* (Hu *et al.*, 2020; Wang *et al.*, 2020) and *Litopenaeus vannamei* (Hu *et al.*, 2019).

Black soldier fly larvae are commonly used in dried or defatted form (Hu *et al.*, 2019, 2020; Li *et al.*, 2017; Wang *et al.*, 2019, 2020) in aquafeeds. However, it is easy to understand that drying and defatting may lead to nutrition loss of larvae and extra costs during commercial processing, such as lipid oxidation caused by drying, or lipid and amino acid loss during defatting, as summarised from results of our previous studies (Hu *et al.*, 2017a,b; Wang *et al.*, 2019, 2021). On the contrary, black soldier fly pulp (BSFP) used in this study was directly made from fresh and clean larvae and its nutrient value is close to the nature. BSFP saved processing technology and equipment and thus saved costs of feed when considering commercial production. For another reason, BSFP is suitable to be used as aquafeed because insects are historically regard as much of the basic prey for omnivorous and carnivorous fish (Henry *et al.*, 2015; Nogales-Merida *et al.*, 2019), although there would also be negative to using BSFP such as the increased transport cost due to weight of water and the decreased durability of the insect ingredient before used in feed. Nevertheless, evaluation about the effects of BSFP on growth and health of fish remain rare. Only a recent study by Xu *et al.* (2020) documented that BSFP at the level below 131 g/kg could be added in diet of juvenile mirror carp (*Cyprinus carpio* var. specularis) without any negative effects on growth performance and intestinal health. However, to our best knowledge, research has not been conducted in carnivorous fish species. Our previous studies in black soldier fly larvae meal showed inconsistent results in growth and antioxidant capacity between omnivorous (*Ctenopharyngodon idellus*) and carnivorous (*L. japonicus*) fish (Huang *et al.*, 2019; Wang *et al.*, 2019), depending on larvae forms and inclusion levels in diets.

Micropterus salmoides, also known as California perch, is one of the most commercially valuable carnivorous species that has been widely cultured worldwide. In 2019, China contributed nearly 500,000 tons of the freshwater farmed *M. salmoides* (China Fishery Statistical Yearbook, 2020). The objective of this study was to evaluate the effects of BSFP on the growth performance, whole body composition, serum metabolites, antioxidant and immune capacity of *M. salmoides*.

2. Materials and methods

Diet preparation

The proximate and nutrient composition of the experimental diets was shown in Table 1 and the chemical composition of BSFP (obtained from Guangzhou Anruijie Environmental Protection Technology Co. Ltd., Guangzhou, China) was as follows: dry matter 40%, crude protein 14%, crude lipid 15%, ash 5% and chitin 2%. The BSFP (original substance) was added to the basal diet at the rate of 0% (BSFP0), 1% (BSFP1), 2% (BSFP2) and 4% (BSFP4), corresponding to inclusion of 0, 4.5, 9.0 and 18.0 g/kg DM in diets. All diets were extruded using the Valva-60 extruder (Guangzhou Valva Machinery Equipment Co. Ltd, Guangzhou, China) into 2 mm pellets, dried at 55 °C overnight and stored at -20 °C.

Table 1. Ingredients and proximate composition of the experimental diets.[1]

Ingredients (g/kg DM)	BSFP0	BSFP1	BSFP2	BSFP4
Fish meal	450	450	450	450
Black soldier fly pulp	0	4.5	9	18
Soy protein concentrate	225	225	225	220
Peeled soybean meal	110	110	110	110
Alpha starch	124	119.5	115	111
Fish oil	40	40	40	40
Soya oil	20	20	20	20
Monocalcium phosphate	15	15	15	15
Vitamin premix[2]	1	1	1	1
Mineral premix[3]	5	5	5	5
Vitamin C ester	3	3	3	3
Choline chloride	5	5	5	5
Methionine	2	2	2	2
Proximate composition				
Dry matter	952	949	947	948
Crude protein	504.2	509.0	510.5	508.5
Crude lipid	94.7	93.9	94.4	96.4
Ash	111	110	110	111

[1] BSFP0-BSFP4, percentage of black soldier fly pulp included in the basal diet were 0, 1, 2 and 4%, respectively.

[2] One kilogram of vitamin premix provided: VA 3,230,000 IU, VD 1,600,000 IU, VE 16 g, VK_3 4 g, VB_1 4 g, VB_2 8 g, VB_6 4.8 g, VB_{12} 0.016 g, nicotinic acid 28 g, pantothenic acid calcium 16 g, biotin 0.064 g, folic acid 1.285 g, inositol 40 g.

[3] One kilogram of vitamin premix provided: Ca 230 g, K 36 g, Mg 9 g, Fe 10 g, Zn 8 g, Mn 1.9 g, Cu 1.5 g, Co 0.25 g, I 0.032 g, Se 0.05 g.

Experimental design and feeding management

The experimental fish were provided by the Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, China). A total of 420 healthy *M. salmoides* (initial body weight 4.9±0.10 g) were randomly distributed into 4 groups with triplicate per group, and assigned to 12 tanks with 35 fish in each tank. Fish were fed to apparent satiation twice daily (07:00 and 19:00) during the 62-d feeding trial. Orts and faeces were siphoned out daily before morning feeding. Feed intake (FI) was calculated as the difference between the amount of feed offered and orts. During the 62-d trial, the water quality was kept at dissolved oxygen >5 mg/l, water temperature 27-30 °C, pH 7.6-7.9, ammonia ≤0.1 mg/l and nitrite ≤0.01 mg/l.

Sampling

Prior to sampling, all fish were fasted for 24 h and then anaesthetised with 40 mg/l of tricaine methanesulfonate. Fish per tank were counted and weighted to determine for final body weight (FBW), weight gain rate (WGR), FI, feed conversion ratio (FCR), and specific growth rate (SGR). Three fish in each tank were randomly collected to analyse for body composition.

Blood was collected from the caudal veins of 15 fish in each tank, samples were pooled per tank and then centrifuged at 3,500 r/min for 10 min. The resultant serum was collected to determine serum parameters. Six fish per tank were randomly collected, slaughtered and measured for condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), intestinesomatic index (ISI), and intraperitoneal fat ratio (IFR).

Laboratory analyses

The nutrient composition of BSFP, diets and fish were determined using AOAC method (AOAC, 1999). Dry matter was analysed by drying samples to a constant weight at 105 °C. Crude protein was measured using the Kjeldahl method. Crude lipid was determined according to the Soxhlet method. Ash was measured by combustion at 550 °C for 6 h. Calcium and total P were determined using method as described by Hu *et al.* (2018). Chitin was analysed as D-glucosamine using the method described by Madrid *et al.* (2013).

Serum metabolites were measured using Hitachi 7180 Biochemical Analyzer (Tokyo, Japan). Antioxidant and immune parameters were determined using commercial kits provided by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Data calculations and statistical analysis

All data were summarised and averaged for each tank and parameters were calculated as follows:

1. WGR (%) = 100 × (FBW (g) – IBW (g)) / IBW (g)
2. FI (g/fish) = feed consumed (g) / (final fish number + initial fish number) / 2
3. FCR = FI (g) / (FBW (g) – IBW (g))
4. SGR (%/d) = 100 × (ln FBW (g) – ln IBW (g)) / days
5. SR (%) = 100 × (final fish number) / (initial fish number)
6. CF (g/cm^3) = body weight (g) / body length^3 (cm)
7. VSI (%) = 100 × visceral weight (g) / body weight (g)
8. HSI (%) = 100 × hepatopancreas weight (g) / body weight (g)
9. ISI (%) = 100 × intestine weight (g) / body weight (g)
10. IFR (%) = 100 × intraperitoneal fat weight (g) / body weight (g)

Tank was used as the statistical unit. All data were subjected to a one-way ANOVA using SPSS 17.0 followed by the Tukey test. Significant differences were declared at $P<0.05$ and the results are shown as means ± SE.

3. Results

Growth performance and body composition

Fish fed BSFP2 had higher ($P<0.05$) FBW, WGR, SGR and IFR than those of fish fed other diets (Table 2). All fish had similar ($P>0.05$) IBW, FI, FCR, SR, CF, VSI, HIS and ISI. The content of CL and ash were higher ($P<0.05$) in fish fed BSFP2 than those of fish fed BSFP0 (Table 3). Fish fed BSFP2 and BSFP4 had higher ($P<0.05$) level of Ca than those of fish fed BSFP0.

Serum metabolites, antioxidant and immune response

Fish had similar ($P>0.05$) total cholesterol, triacylglycerol, albumin, globulin, glucose, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, low density lipoprotein cholesterol and high density lipoprotein cholesterol(Table 4), and similar total antioxidant capacity, catalase (CAT), glutathione peroxidase, superoxide dismutase, alkaline phosphatase and lysozyme regardless of the treatments (Table 5). Fish fed BSFP2 and BSFP4 had lower ($P<0.05$) malondialdehyde (MDA) but higher ($P<0.05$) acid phosphatase (ACP) than those of fish fed other diets.

4. Discussion

Growth performance and body composition

Black soldier fly larvae have been shown an important ingredient in aquafeed, whereas little information is available about the effects of BSFP on growth and health of fish. The similar feed intake and survival rate of fish among diets in this study suggested that BSFP was palatable for *M. salmoides*. Similar results have been observed when BSFP was included in the mirror carp (*C. carpio* var. specularis) diets (Xu *et al.*, 2020). Significantly increased weight gain rate and specific growth rate in BSFP2 as compared with other diets indicated that dietary inclusion of 9 g/kg DM BSFP improved growth performance of *M. salmoides*. Previous studies have been well documented that black soldier fly larvae could be partly or fully replace fishmeal in aquafeeds without impairing growth performance of rainbow trout (*Oncorhynchus mykiss*) (Renna *et al.*, 2017; Sealey *et al.*, 2011), Atlantic salmon (*Salmo salar*) (Lock *et al.*, 2016), European seabass (*Dicentrarchus labrax*) (Magalhaes *et al.*, 2017), Pacific white shrimp (*L. vannamei*) (Cummins *et al.*, 2017; Hu *et al.*, 2019), Jian carp (*C. carpio var.* Jian) (Zhou *et al.*, 2017), tilapia (Muin *et al.*, 2017), koi cap (*C. carpio*) (Liu *et al.*, 2017a), yellow catfish (*P. fulvidraco*) (Hu *et al.*, 2017a; Wang *et al.*, 2020; Xiao *et al.*, 2018) and Japanese seabass (*L. japonicus*) (Hu *et al.*, 2018; Wang *et al.*, 2019). Xu *et al.* (2020) also reported that up to 174.7 g/kg DM BSFP in diets had no negative effect on growth performance of mirror carp (*C. carpio* var. specularis). However, Cummins *et al.* (2017) found

Table 2. Effect of inclusion of black soldier fly pulp (BSFP) on growth performance of *Micropterus salmoides*.[1,2]

	BSFP0	BSFP1	BSFP2	BSFP4
IBW (g)	4.89±0.01	4.89±0.01	4.90±0.01	4.89±0.01
FBW (g)	56.71±1.66[b]	58.27±1.12[b]	64.01±0.90[a]	59.02±1.85[b]
WGR (%)	1,059.71±34.31[b]	1,091.62±22.97[b]	1,206.33±19.66[a]	1,107.20±37.59[b]
FI (g/fish)	39.96±1.96	41.06±0.77	42.69±1.16	39.98±0.85
FCR	0.77±0.01	0.77±0.02	0.72±0.02	0.74±0.01
SGR (%)	3.95±0.05[b]	3.99±0.03[b]	4.15±0.02[a]	4.01±0.01[b]
SR (%)	94.30±3.29	90.57±2.87	97.13±1.65	97.13±1.65
CF (g/cm^3)	2.40±0.13	2.44±0.15	2.44±0.18	2.39±0.11
VSI (%)	6.94±0.90	7.35±0.98	7.63±1.16	7.51±1.04
HSI (%)	2.42±0.88	2.66±0.77	2.59±0.71	2.77±0.84
ISI (%)	0.56±0.11	0.60±0.09	0.61±0.08	0.56±0.07
IFR (%)	1.34±0.31[b]	1.47±0.37[b]	1.81±0.45[a]	1.57±0.28[ab]

[1] BSFP0-BSFP4, percentage of black soldier fly pulp included in the basal diet were 0, 1, 2 and 4%, respectively. CF = condition factor; FBW = final body weight; FCR = feed conversion ratio; FI = feed intake; HIS = hepatosomatic index; IBW = initial body weight; IFR = intraperitoneal fat ratio; ISI = intestinesomatic index; SGR = specific growth rate; SR = survival rate; VSI = viscerosomatic index; WGR = weight gain rate.

[2] Means with different letters differ ($P<0.05$).

Table 3. Effect of inclusion of black soldier fly pulp (BSFP) on body composition (% original substance) of *Micropterus salmoides*.[1,2]

	BSFP0	BSFP1	BSFP2	BSFP4
DM	27.91±0.67	28.02±0.21	29.05±0.35	28.64±0.45
CP	17.26±0.11	17.57±0.48	17.86±0.40	17.61±0.23
CL	4.90±0.51[b]	5.96±0.18[ab]	6.34±0.11[a]	5.99±0.42[ab]
Ash	3.27±0.06[b]	3.54±0.15[ab]	3.75±0.13[a]	3.65±0.14[ab]
Ca	0.70±0.02[b]	0.86±0.04[ab]	0.96±0.08[a]	0.94±0.08[a]
Total P	0.61±0.06	0.54±0.07	0.68±0.02	0.62±0.02

[1] BSFP0-BSFP4, percentage of black soldier fly pulp included in the basal diet were 0, 1, 2 and 4%, respectively. Ca = calcium; CL = crude lipid; CP = crude protein; DM = dry matter; P = phosphorus.

[2] Means with different letters differ ($P<0.05$).

Table 4. Effect of inclusion of black soldier fly pulp (BSFP) on serum metabolites of *Micropterus salmoides*.[1]

	BSFP0	BSFP1	BSFP2	BSFP4
TCHO (mmol/l)	7.57±0.80	7.78±0.54	6.30±0.14	7.69±0.63
TG (mmol/l)	2.65±0.08	3.22±1.01	2.17±0.09	2.86±0.31
ALB (g/l)	8.80±0.17	9.33±0.49	10.53±0.27	9.50±1.06
GLB (g/l)	14.90±0.97	16.50±0.78	16.37±0.43	15.20±0.26
GLU (mmol/l)	6.24±0.65	4.76±0.82	6.18±0.19	5.63±1.11
BUN (mmol/l)	4.27±0.57	3.67±0.07	3.53±0.33	3.93±0.07
AST (U/l)	122.33±6.84	120.33±12.14	125.33±8.09	116.33±6.74
ALT (U/l)	1.67±0.33	2.00±0.58	2.00±0.00	1.67±0.33
LDLC (mmol/l)	2.07±0.39	2.13±0.26	1.92±0.02	2.33±0.39
HDLC (mmol/l)	1.59±0.11	1.59±0.20	1.54±0.02	1.57±0.09

[1] BSFP0-BSFP4, percentage of black soldier fly pulp included in the basal diet were 0, 1, 2 and 4%, respectively. ALB = albumin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; GLB = globulin; GLU = glucose; HDLC = high density lipoprotein cholesterol; LDLC = low density lipoprotein cholesterol; TCHO = total cholesterol; TG = triacylglycerol.

Table 5. Effect of inclusion of black soldier fly pulp (BSFP) on serum antioxidant and immune response of *Micropterus salmoides*.[1,2]

	BSFP0	BSFP1	BSFP2	BSFP4
Antioxidant				
TAOC (U/ml)	12.88±0.74	11.34±0.83	12.50±0.50	10.97±0.20
CAT (U/ml)	5.45±1.43	5.81±0.25	4.92±0.32	5.33±0.22
GPx (U/ml)	2,504.51±48.91	2,674.26±34.36	2,846.46±44.39	2,666.41±78.45
SOD (U/ml)	173.62±21.55	159.44±10.58	198.14±1.75	168.60±9.45
MDA (nmol/ml)	9.82±0.54[a]	9.66±0.78[a]	7.62±0.96[b]	5.29±0.54[c]
Immune (U/ml)				
AKP	14.67±1.22	13.87±1.02	12.64±0.60	13.82±0.97
ACP	7.25±0.09[b]	7.37±0.14[b]	7.84±0.12[a]	7.74±0.05[a]
LZM	71.11±5.51	77.17±5.85	71.56±3.62	67.98±1.47

[1] BSFP0-BSFP4, percentage of black soldier fly pulp included in the basal diet were 0, 1, 2 and 4%, respectively. ACP = acid phosphatase; AKP = alkaline phosphatase; CAT = catalase; GPx = glutathione peroxidase; LZM = lysozyme; MDA = malondialdehyde; SOD = superoxide dismutase; TAOC = total antioxidant capacity.

[2] Means with different letters differ ($P<0.05$).

that inclusion of black soldier fly larvae in shrimp diets depressed growth performance when the replacement rate of fishmeal by black soldier fly larvae meal exceeded 25%. Wang *et al.* (2020) observed decreased growth performance of yellow catfish (*P. fulvidraco*) when the replacement rate of fishmeal by black soldier fly larvae meal exceeded 40%. In this study, reduced growth performance observed in BSFP4 was most likely due to increased chitin as dietary BSFP increasing. Chitin was reported to inhibit nutrient absorption resulting in reduced growth performance of fish (Shiau and Yu, 1999). Even lower content of chitin (1%) can decrease growth performance of carp and Atlantic salmon (Gopalakannan and Arul, 2006; Olsen *et al.*, 2006). In summary, these discrepancies observed on the effects of black soldier fly larvae on growth performance among studies may have resulted from variations in diet formulations and/or differences in animal species.

It is interesting to note that dietary inclusion of 9 g/kg DM BSFP increased the intraperitoneal fat ratio and crude lipid content of *M. salmoides*, suggesting that BSFP could promote fat deposition. This is unlikely attributed to nutrient levels of diets because all diets in this study were formulated to be isoproteic and isolipidic. Our previous studies observed that dietary inclusion of full fat or defatted black soldier fly larvae meal did not alter fat deposition of fish (Wang *et al.*, 2019) or even inhibit fat deposition (Wang *et al.*, 2020). However, research on BSFP remain rare. A recent study by Xu *et al.* (2020) reported that dietary inclusion of BSFP up to 174.7 g/kg DM had no influence on the intraperitoneal fat index but decreased whole body crude lipid content of mirror carp (*C. carpio* var. specularis). Differences between this study and Xu *et al.* (2020) may due to variations in fish species or differences in BSFP inclusion levels. The inclusion level of BSFP is 4.5-18.0 g/kg DM in this study, which is much lower than 43.7-174.7 g/kg DM in Xu *et al.* (2020). Furthermore, black soldier fly larvae contain high level of saturated and monounsaturated fatty acids which is quite different from fishmeal. It has been reported that high level of saturated and monounsaturated fatty acids is usually conducive to fat accumulation (Yi, 2018). Moreover, the larvae used in this study reared on kitchen waste substrate had higher crude lipid content than those of larvae fed wheat bran (Xu *et al.*, 2020) or other substances (Hu *et al.*, 2017b). Yi (2018) reported that increased inclusion level of black soldier fly larvae oil in yellow catfish (*P. fulvidraco*) diets increased the saturated and monounsaturated fatty acid deposition. In this study, the increased whole body crude lipid content in BSFP2 is most likely attribute to correspondingly increased intraperitoneal fat ratio. Increased whole body crude lipid content by inclusion of black soldier fly larvae in diets has also been reported in shrimp (*L. vannamei*) (Hu *et al.*, 2019) and Japanese seabass (*L. japonicus*) (Hu *et al.*, 2018). Similarly, increased whole body ash and Ca levels in BSFP2 as compared with BSFP0 suggested that inclusion of black soldier fly larvae in *M. salmoides* diets promote Ca deposition. This is consistent with our previous observation by Hu *et al.* (2019, 2020). Furthermore, the increased intraperitoneal fat along with increased body composition of fish fed BSFP treated diets may partly account for improved growth performance of *M. salmoides*.

Serum antioxidant and immune response

The similar serum metabolites among diets suggests dietary inclusion of BSFP up to 18 g/kg DM did not adversely impact on these metabolite profiles. However, decreased MDA concentration as dietary BSFP increasing indicated that inclusion of BSFP may enhance the antioxidant capacity of *M. salmoides*. MDA is a product of polyunsaturated fatty acid peroxidation and directly reflects the degree of endogenous oxidative damage (Ding *et al.*, 2015). Buege and Aust (1978) reported that increased MDA can result in tissue damage. Decreased serum MDA concentration has also been observed in Jian carp (*C. carpio* var. Jian) fed black soldier fly larvae meal (Zhou *et al.*, 2017) and Japanese seabass (*L. japonicus*) fed defatted black soldier fly larvae meal (Wang *et al.*, 2019). A recent study by Xu *et al.*, (2020) reported that dietary inclusion of BSFP improved antioxidant status of mirror carp (*C. carpio* var. specularis) by increasing serum CAT activity and decreasing MDA concentration. Improved antioxidant status of *M. salmoides* by inclusion of BSFP in this study is likely due to the antioxidant effect of chitin. Kroeckel *et al.* (2012) reported that black soldier fly larvae contain polysaccharide chitin in their exoskeleton. Chitin was found to have antioxidant properties (Ngo and Kim, 2014; Ngo *et al.*, 2009). ACP is an important enzyme of animal lysosomes (Blasco *et al.*, 1993). This study showed that dietary inclusion of BSFP at 9 g/kg DM increased serum ACP activity, suggesting that BSFP may enhance immune capacity of *M. salmoides*. This is consistent with observation by Xiao *et al.* (2018) in yellow catfish (*P. fulvidraco*) that dietary inclusion of black soldier fly larvae meal improved immune capacity of fish, owing the contribution to the presence of chitin in the larvae. Another reason for the increased antioxidant and immune capacity by BSFP in this study is likely due to increased lauric acid as dietary BSFP increasing. Black soldier fly was reported to contains 19.2 and 9.7% of lauric acid (% of total fatty acid) from insect oil (Hu *et al.*, 2020) and insect meal (Hu *et al.*, 2017b), respectively. Lauric acid or its derivative glycerol monolaurate has been shown to have an effect as antioxidant and immune modulator (Liu *et al.*, 2017b; Sun *et al.*, 2021) owing to its activation on antioxidant and immune system. Also, vitamins, peptides, or other antioxidants not known or described in the insect pulp may contribute to the improved antioxidant and immune capacity although these active substances have not been determined in this study. Further study is still needed to confirm this.

5. Conclusions

Dietary inclusion of 2% BSFP improved growth performance of *M. salmoides* might be attributed to increased lipid and ash deposition. BSFP improved antioxidant and immune capacity but also increased intraperitoneal fat deposition of fish, suggesting BSFP should be careful to be used in *M. salmoides* diets.

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Conflicts of interest

The authors declare no conflict of interest.

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