DRF-422
[1-8]

RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Optimizing Black Soldier Fly Larva Meal Substitution for Growth Performance and Meat Quality of Loach Fish

Nhien Thi Nguyen[1], Nam Hoang Tran[2]

10.18805/ajdfr.DRF-422

ABSTRACT

Background: The study was conducted to evaluate the substitution of fishmeal by black soldier fly larvae (BSFL) meal on growth performance, survival rate, feed utilization and meat quality of loach fish.

Methods: The experimental fish, weighing between 3 g and 8 g, were randomly assigned to one of four groups, treated with 0% (CG), 10% (TG1), 30% (TG2) and 50% (TG3) BSFL-substituted fishmeal.

Result: The growth performance in terms of length and weight was significantly higher in the TG1 and TG2 compared to the other groups. We also confirmed a positive correlation between the length (x) and weight (y) of loach fish. Regarding the quality of meat, TG1 exhibited the highest amino acid content at 18.52%. However, survival rate and feed utilization across all treatments showed no significant differences.

Key words: Black soldier fly larvae, Growth performance, Loach fish, Meal substitution.

INTRODUCTION

As the global population continues to grow, the demand for protein sources is on the rise, leading to an increasing prominence of fish farming. However, the continuous and excessive exploitation of fish resources worldwide has resulted in depletion. To address this concern, novel techniques are being adopted to bolster production in the aquaculture industry.

The utilization of fishmeal, well-recognized for its exceptional nutritional properties, occupies a central role in the formulation of fish feed. Nevertheless, challenges associated with economic feasibility and availability have precipitated the exploration of alternative protein sources aimed at replacing fishmeal. Within this context, insect-based protein has emerged as a focal point of investigation, undergoing rigorous experimental feeding trials that have positioned it as a highly promising substitute (Milesh *et al.* 2024). Insects such as the black soldier fly (*Hermetia illucens*) (Alqazzaz *et al.* 2019), house flies, flesh flies (Sharawi, 2015), grasshoppers and mealworms have garnered considerable attention due to their abundant protein, lipid, vitamin and mineral content, coupled with the minimal presence of anti-nutritional factors. The ease with which they can be cultivated and their capacity to yield substantial biomass with a commendable nutritional profile further distinguish them as preferred sources of animal protein. Remarkably, incorporating modest levels of insect meal substitution (around 25-30%) into fish feed has shown notable enhancements in growth rate, survival and immunity (Tippayadara *et al.,* 2021; Boakye-Yiadom, Ilari and Duca 2022). This underscores the potential of insect-based protein as a valuable component in advancing the aquaculture sector.

In recent times, there has been a surge in interest surrounding insect-derived protein sources, particularly those sourced from black soldier flies (BSF). This interest arises from their capacity to thrive on organic waste, a characteristic that has been gaining attention. Notably, BSFs require much less feed due to their lower greenhouse gas emissions when compared to cattle (Boakye-Yiadom, Ilari and Duca, 2022). The efficiency of insect reproduction, facilitated on a smaller waste surface, results in a higher yield per hectare compared to other crops (Verbeke *et al*., 2015). The nutritional composition of these insects is commendable, encompassing essential and non-essential amino acids, both saturated and unsaturated fatty acids, vitamins, minerals, polysaccharide polymers, bioactive compounds, antioxidants, high feed conversion ratios (FCR), as well as antibacterial and immunostimulant factors (Harikrishnan *et al.* 2012). Recent research has further broadened the applications of BSFL, demonstrating their benefits beyond aquaculture. BSFL supplementation has been shown to enhance meat yield and quality in Ac

[1]Faculty of Biotechnology, Vietnam National University of Agriculture, Trau Quy Ward, Gia Lam District, Hanoi 131000, Vietnam.
[2]Research Center for Higher Education, Tokushima University, 1-1 Minami-Josanjima, Tokushima 770-8502, Japan.

Corresponding Author: Nam Hoang Tran, Research Center for Higher Education, Tokushima University, 1-1 Minami-Josanjima, Tokushima 770-8502, Japan. Email: tran@tokushima-u.ac.jp
ORCIDs: 0000-0001-9361-3217, 0000-0002-9985-5628.

How to cite this article: Nguyen, N.T. and Tran, N.H. (2025). Optimizing Black Soldier Fly Larva Meal Substitution for Growth Performance and Meat Quality of Loach Fish. Asian Journal of Dairy and Food Research. 1-8. doi: 10.18805/ajdfr.DRF-422.

Submitted: 02-08-2024 **Accepted:** 25-01-2025 **Online:** 24-02-2025

chickens (Nguyen and Tran, 2024). Additionally, studies on the genetic diversity of BSFL populations in Vietnam have provided crucial insights for optimizing their use in animal feed and other industries (Nguyen *et al*., 2024).

Loach (*Misgurnus anguillicaudatus*), a freshwater fish species of considerable economic, nutritional and medicinal value, has gained popularity among Vietnamese cuisine. In traditional Chinese medicine, loach fish has been employed for the treatment of ailments such as hepatitis, osteomyelitis, ulcers, cancer and convalescence in elderly individuals (Qin *et al*., 2002). Displaying remarkable adaptability to various environments, loach fish has been cultivated through diverse farming techniques, responding to market demands and favorable natural conditions, thus leading to an increase in commercial loach farming.

In the context of aquafeed production, fishmeal has long held the status of a premium protein source. However, extensive and large-scale fishing for this purpose has adversely impacted marine ecosystems and the environment, resulting in the depletion of fishmeal resources. Escalating scarcity and elevated prices have consequently affected aquaculture farmers. Research trials have substantiated that incorporating insect meal into fish diets does not compromise growth rates or body mass (Zhou *et al.,* 2018).

While past studies have explored the substitution of fishmeal with BSF larvae (BSFL) in the diets of aquatic animals, the specific substitution and optimal ratio of fishmeal with BSFL has not been thoroughly investigated. As a result, our study aims to examine the effects of substituting fishmeal with BSFL in the diet on growth performance and the quality of fish meat.

MATERIALS AND METHODS

Materials

The loach fish individuals ranging from 3 to 8 grams per fish were procured from the hatchery of the Research Institute of Aquaculture I - Bac Ninh province. Prior to initiating the experiment, a size and weight-based classification of the fish was conducted, meticulously allocating them into experimental batches. This allocation aimed to achieve equitable food distribution for every individual within each batch, consisting of 30 fish with similar characteristics (size ranging from 3 to 8 grams per individual), considering the fish's aggressive behavior during feeding, as reported previously (Nguyen, 2014).

The experiment was strictly organized, comprising of three distinct treatment groups (TG) and one control group (CG). The feed formula utilized in this study was previously reported (Nguyen, 2014). The ingredients of the experimental diet are described in Table 1. Variations within the experimental formulas were exclusively attributed to the differing percentage compositions of fishmeal, which was sourced from An Hai Investment Joint Stock Company. Notably, the amino acid composition was assayed through sampling and analysis at the Laboratory of Food Science and Technology, Faculty of Food Technology, VNUA. The entire study adhered to the farming protocols stipulated by the Aquaculture Research Institute No. 1.

The feed used in this study was produced using a mechanical pelletizing method, forming pellets of 2,5-3 cm in diameter to suit the feeding behavior of the loach fish (bottom feeder). Ingredients for the feed included fishmeal, BSFL powder, soybean meal, rice bran and cornmeal. The formulations were enriched with a premix of vitamins and minerals, including vitamin C, vitamin E, a range of B-complex vitamins, as well as essential trace elements such as zinc and selenium to support fish health and growth. To enhance pellet stability and reduce disintegration in water, binders such as carboxymethyl cellulose (CMC) were added.

The research was conducted in 2024, utilizing BSFL cultivated in the Animal Biotechnology Laboratory at VNUABSFL used for the experiments was cultivated in the Animal Biotechnology Laboratory at VNUA. The BSFL powder was manufactured following the method described in literature (Boushy and Poel, 2000). The larvae were nourished with processed beans and vegetables procured from a local market. Upon reaching the fifth instar, the larvae underwent a thorough washing process with clean water, followed by drying in a heater set to a temperature of 80 degrees Celsius until achieving a crispy consistency (approximately 4 hours). Subsequently, they were ground into powder and securely sealed in plastic bags. Table 2

Table 1: Fish meal to BSFL substitution ratios in each experimental group (%).

Ingredient (%)	CG	TG1 (10%)	TG2 (30%)	TG3 (50%)
Fish meal	56.9	46.9	26.9	6.9
Soy flour	28.4	28.4	28.4	28.4
Rice bran	7.7	7.7	7.7	7.7
Premix vitamins - minerals*	2	2	2	2
Soybean Oil	2	2	2	2
Adhesives**	3	3	3	3
BSFL powder	0	10	30	50
Total	100	100	100	100

* vitamin C, vitamin E, B-complex vitamins, zinc and selenium to support fish health and growth.

** Carboxymethyl cellulose (CMC).

shows the comparison of content of amino acids in fishmeal and BSFL for comparison.

Experiment design

The experiment consisted of three distinct treatment groups and a control group, arranged in a fully randomized design, with 3 replicates, including: CG (without BSFL), TG1 (replacing 10% of fishmeal with BSFL), TG2 (replacing 30% of fish meal with BSFL), TG3 (replacing 50% of fish meal with BSFL). The food was prepared by mixing ingredients in defined proportions, forming pellets, then stored in refrigerator.

Evaluation criteria

Monitored metrics include:
The total length of each fish individual was recorded with a 150mm Syntek Carbon fiber electronic caliper and then analyzed the growth rate according to the formula:
Daily length growth rate (DLG):

$$\text{DLG (mm/day)} = \frac{\text{Lb - Le}}{\text{t}}$$

Specific length growth rate (SGR):

$$\text{SGR (\%/day)} = \frac{\text{lnLe - lnLt}}{\text{t}} \times 100$$

Where:
Lb = Original length.
Le = Length at the end of the experiment.
T = Experimental time.

The total weight of each fish individual was recorded with a Metamo WH-B05 electronic compact scale, then growth speed is calculated by the formula:

Daily weight growth (DWG):

$$\text{DWG (g/day)} = \frac{\text{We - Wb}}{\text{t}}$$

Specific weight growth rate (SGR):

$$\text{SGR (\%/day)} = \frac{\text{lnWe - lnWb}}{\text{t}} \times 100$$

Where:
Wb = Initial length.
We = length at the end of the experiment.
T = Experimental time.

The oxygen required for fish rearing was delivered by the Super Pump SI-2800 oxygen system. Oxygen saturation levels were assessed using a handheld EZ-DO 7031 meter and a Hana HI98107 pH tester.

On the 60th day, the loach fish were harvested and subjected to various analyses, including measurements of growth rate in terms of length and weight, determination of survival rate, calculation of the feed conversion ratio and assessment of amino acid content in the meat following 60 days of rearing.

The relationship between loach fish length (x) and weight (y) was established using the linear regression equation:

$$y = ax + b$$

This length-weight relationship is crucial for evaluating the functional and ecological implications for fish species. Additionally, the coefficients ‘a’ and ‘b’ are valuable for estimating individual weight from length data and defining the condition index, thereby aiding in the assessment of population morphology in different ecological regions.

Data processing

The data were organized using Excel software. Statistical analysis was conducted through an ANOVA test of Minitab software. The correlation between fish length and weight was determined using the regression feature within Minitab software.

RESULTS AND DISCUSSION

Effect of BSFL on the length and weight growth of loach fish

The growth in the length of fish was periodically measured, processed and analyzed during the experiment. Table 3 shows that the growth in length of TG1, TG2 and TG3 has a difference compared with the CG ($p<0.05$). After 60 days of rearing, fish achieved the best average growth in length in the CG treatment, which was 19.60±2.28 mm/fish in comparison with the initial length, while TG3 had the lowest growth in length at 11.21±0.25 mm/fish. This proves that replacing fishmeal with BSFL with 10% - 30% content did not affect the growth of fish in length. However, if not

Table 2: Content (%) of amino acids in fishmeal and in BSFL*.

Criterion	Fishmeal (%)	BSFL (%)
	Essential amino acids	
Methionine	1.75	0.95
Histidine	1.73	0.48
Threonine	2.04	1.18
Valine	2.80	1.78
Phenylalanine	2.59	1.34
Isoleucine	2.73	1.21
Leucine	4.05	1.93
Lysine	4.49	1.62
Total	22.18	10.49
	Non-essential amino acids	
Alanine	2.34	1.91
Tyrosine	1.83	1.71
Glutamate	-	3.91
Serine	1.85	1.27
Cystine	-	0.02
Glycine	3.67	1.97
Aspartic acid	5.85	2.82
Arginine	-	1.18
Proline	2.77	1.46
Total	18.54	16.25
Total amino acids	40.72	26.74

* Analysis conducted at the laboratory of the faculty of food engineering, VNUA.

supplemented with BSFL or with more than 30% of BSFL, it will affect the growth of fish.

The results of DLG monitoring showed that the fish had the highest length growth rate in the CG at 0.33 ± 0.04 mm/day and the lowest in TG3 at 0.19±0.01 mm/day. We found TG2 and TG3 had a difference with CG ($p<0.05$), but TG1 had no difference with CG. One possible explanation for this result is that the BSFL is not only rich in protein, but its chitin content is also higher than that of the sea fish powder (Makkar *et al.* 2014). Besides, feed with a high content of chitin negatively affects the passive nutrient absorption and nutrient digestibility of fish (Kroeckel *et al.,* 2012).

Our results are entirely consistent with the results of a previous study (Cummins *et al.,* 2017). Another study has been conducted about substitution of fishmeal protein with soymeal protein in feed, showed that the growth of seabass (*Lates calcarifer*) decreased when the substitution level exceeded 10% (Tantikitti, Sangpong and Chiavareesajja 2005). Similarly, when replacing more than 20% of fishmeal protein with soymeal protein in the feed of spotted snapper (*Lutjanus guttatus*), growth was also reduced (Silva-Carrillo *et al.,* 2012).

Weight growth of fish was periodically measured and analyzed during the experiment. Table 4 shows that fish cultured in all experimental groups had linear growth. After 60 days of rearing, the average growth of the fish was 8.48 - 11.37 g/individual. In which, the best weight gain was TG1 with an increase of 6.27±0.11 g/individual and the lowest growth was TG3 with an increase of 4.86 0. 31 g/individual. ANOVA analysis showed that the growth in weight of TG1, TG2 and TG3 after 60 days of culture were all significantly different from CG (< 0.05). This proves that the BSFL substitution to the fishmeal affects the weight growth of fish. The results of DWG daily growth monitoring showed that the fish had the highest weight growth rate in TG1 with 0.10 0.01 g/day and the lowest in TG3 0.08 0.00 g/day. TG3 has a statistically significant difference with the CG, TG1 and TG2 (<0.05). However, TG1 and TG2 were not found to have differences compared with the control group. These results were higher than the results of a previous study (Luong and Tran, 2022) using BSFL to replace fishmeal in

Table 3: Effect of BSFL substitution on average length of fish (mm/individual).

Day	Experimental group			
	CG (0%)	TG1 (10%)	TG2 (30%)	TG3 (50%)
0	71.68±0.58	77.06±0.19	80.14±0.65	88.69±0.14
10	72.76±0.75	77.63±0.67	80.90±0.70	89.31±0.16
20	73.31±1.18	80.09±0.26	81.77±0.77	90.66±0.53
30	76.40±0.89	82.72±0.55	83.90±0.90	91.72±0.98
40	78.62±1.30	88.94±0.53	89.79±0.34	93.85±0.84
50	84.62±0.88	90.74±0.46	92.11±2.09	96.63±0.66
60	91.21±2.69	95.36±0.58	95.91±1.54	99.90±0.11
Increase length (mm)	19.60a±2.28	18.29ab±0.74	15.77b±0.98	11.21c±0.25
DLG (mm/ day)	0.33a±0.04	0.31ab±0.02	0.26b±0.02	0.19c±0.01
SGR (%/ day)	0.40a±0.04	0.36ab±0.02	0.30b±0.02	0.20c±0.01

The values presented mean±standard deviation (SD). values within the same row that are denoted by different letters (a, b, c) indicate a statistically significant difference ($p<0.05$).

Table 4: Effect of BSFL substitution on fish weight (g/individual).

Day	Experimental group			
	CG (0%)	TG1 (10%)	TG2 (30%)	TG3 (50%)
0	3.03±0.06	4.29±0.10	4.98±0.40	6.51±0.12
10	4.24±0.05	5.29±0.05	5.82±0.65	7.33±0.47
20	4.88±0.17	5.99±0.05	6.32±0.49	7.46±0.14
30	5.48±0.30	7.08±0.30	7.35±0.58	8.15±0.18
40	6.70±0.46	7.82±0.22	8.44±0.75	9.48±0.14
50	7.32±0.12	8.99±0.06	9.33±0.80	10.24±0.20
60	8.48±0.33	10.56±0.14	10.41±0.26	11.37±0.34
Weight increase from the original (g)	5.45a±0.38	6.27b±0.11	5.43b±0.15	4.86b±0.31
DWG (g/ day)	0.09a±0.01	0.10ab±0.01	0.09ab±0.00	0.08b±0.00
SGR (%/ day)	1.71a±0.09	1.50b±0.03	1.23c±0.09	0.93d±0.05

The values presented mean±standard deviation (SD). Values within the same row that are denoted by different letters (a, b, c) indicate a statistically significant difference ($p<0.05$).

the diet of eels. The current study revealed that when using less than 30% of BSFL supplementation, it did not affect the weight gain of fish. Moreover, the results of the current study are consistent with another study (Tippayadara *et al.,* 2021), which showed that the 60% - 80% BSFL substitution instead of fishmeal in the diets of tilapia, the ability to gain weight was significantly reduced. This can be explained by the fact that the addition of a lot of BSFL has affected the attractiveness of the feed, reducing the fish's ability to take the feed.

Relationship between length and weight

Fig 1 shows the typical growth trajectory of loach fish, elucidating the connection between their length and body weight. We employed a linear regression model to ascertain the association between fish length (x, measured in millimeters) and weight (y, measured in grams). Regression analysis has yielded the following equation characterizing the relationship between length and weight:

$$y = -14.59 + 0.2554x \ (R^2 = 0.904)$$

The derived equation underscores a positive correlation between body length and weight, signifying that knowledge of the fish's length can serve as a predictive indicator for its weight and vice versa.

Effects of BSFL on survival rate and feed conversion ratio of loach fish

The survival rate of the fish in different treatment groups is shown in Table 5. When replacing fishmeal protein with BSFL protein with different concentrations, the survival rate of the fish is quite high. ANOVA analysis showed that there was no statistically significant difference between the four groups, >0.05). This proves that the BSFL substitution to replace fishmeal did not affect the survival rate of fish.

The present study has exhibited a notably superior survival rate in comparison to a prior study (Nguyen and Nguyen, 2016), which employed BSFL substitution for feeding snakehead fish. Notably, their research disclosed that when substituting fishmeal with BSFL in the diet at levels ranging from 10% to 30%, no discernible impact on survival rates was observed. However, when substituting 40% to 50% of the BSFL for fishmeal, the appetite of snakehead fish exhibited a notable reduction. Our findings align with a previous study (Newton *et al.,* 2005) which integrated larvae meal into the diet of catfish and determined that substitution up to 30% with BSFL did not adversely affect survival rates. This observation can be elucidated by the rich composition of biologically active compounds present in larval worms, encompassing

Table 5: Effect of BSFL substitution on survival rate (%).

Criteria	Experimental group			
	CG (0%)	TG1 (10%)	TG2 (30%)	TG3 (50%)
Number of fish stocked initially (individuals)	90	90	90	90
Number of fish harvested (individuals)	84	82	83	82
Survival rate after 60 days (%)	93.33a±0.00	91.11a±1.92	92.22a±1.92	91.11a±1.92

Values within the same row that are denoted by different letters (a, b, c) indicate a statistically significant difference (p<0.05).

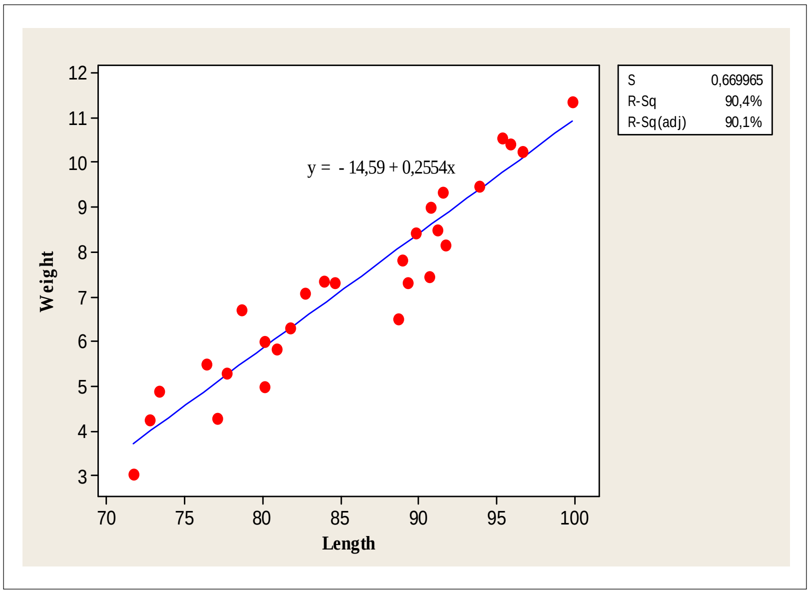

Fig 1: Correlation between length and weight of loach fish.

diverse molecules with various medicinal properties, such as antiviral and antibacterial agents, as well as immune-enhancing capabilities in livestock, poultry and aquatic species, as previously reported (Nguyen *et al.* 2019). Nevertheless, it is worth noting that our results did not attain the same level of efficiency as a study conducted in Japan, which reported a marked enhancement in growth rates, survival rates and feed efficiency upon integrating insect substitution into the diet of red sea bream (Ido *et al.* 2015).

Table 6 presents the feed conversion ratio (FCR) data. The findings delineated in Table 7 demonstrate that there is no statistically significant variance in the feed conversion coefficients between the CG and TG1, TG2, TG3 ($p>0.05$). Consequently, it can be inferred that the substitution of fishmeal protein with BSFL at varying levels within the diet does not exert a discernible influence on the feed conversion ratio.

Notably, the current investigation attains a superior FCR when compared to the study conducted on snakehead fish (Nguyen and Nguyen, 2016). It is noteworthy that the author posited that the substitution of less than or equal to 30% of fishmeal with BSFL in the dietary regimen does not engender any consequential alteration in FCR; however, when the substitution exceeds 30%, it is associated with an elevation in the FCR of the fish. Several other investigations, such as those conducted on salmon (St-Hilaire *et al.,* 2007) and on catfish (Bondari and Sheppard, 1987), have reported that it is feasible to substitute at least 25% of fishmeal protein with red-legged earthworm larvae meal in the diets of the aforementioned species without inducing any significant perturbation in their FCR.

Effects of BSFL on the quality of fish meat

The human body needs two distinct categories of amino acids to facilitate its growth and vital physiological functions. These amino acids are classified into two principal types: essential amino acids, which are incapable of endogenous synthesis within the body and non-essential amino acids, which can be synthesized internally. Within the context of current research, an exhaustive analysis of 17 amino acids in fish meat was conducted (Table 7).

The outcomes revealed that the aggregate amino acid content, encompassing both essential and non-essential amino acids, was most abundant in TG1, constituting

Table 6: Effect of larval meal substitution on fish feed conversion ratio (%).

Criteria	Experimental group			
	CG (0%)	TG1 (10%)	TG2 (30%)	TG3 (50%)
Total amount of feed (g)	205	210	225	231
Total amount of fish harvested (g)	855	876	939	964
FCR	0.39a±0.09	0.41a±0.06	0.50a±0.07	0.48a±0.12

Values within the same row that are denoted by different letters (a, b, c) indicate a statistically significant difference ($p<0.05$).

Table 7: Amino acid content in fish meat (%).

Criteria	CG (n = 30)	TG1 (10%) (n = 30)	TG2 (30%) (n = 30)	TG3 (50%) (n = 30)
Essential amino acids				
Methionine	1.26	1.38	1.23	1.10
Histidine	0.94	0.74	0.79	0.71
Threonine	0.95	1.05	0.93	0.83
Valine	0.79	0.78	0.73	0.65
Phenylalanine	0.84	0.92	0.82	0.73
Isoleucine	0.89	0.96	0.86	0.77
Leucine	0.46	0.48	0.44	0.39
Lysine	1.07	1.13	1.02	0.92
Total	7.20	7.44	6.81	6.09
Non-essential amino acids				
Alanine	0.49	0.53	0.47	0.42
Tyrosine	1.47	1.53	1.39	1.25
Glutamate	2.25	2.16	2.06	1.84
Serine	1.11	1.15	1.05	0.94
Cystine	1.80	1.96	1.75	1.56
Glycine	0.89	0.97	0.86	0.77
Aspartic acid	1.58	1.38	1.38	1.24
Arginine	0.34	0.37	0.33	0.29
Proline	0.95	1.03	0.92	0.82
Total	10.88	11.08	10.22	9.15

18.52% of the total fish weight, while it was least abundant in TG3, representing 15.24% of the fish weight. Notably, across all treatment groups, the feed formulations consistently comprised eight essential amino acids, namely Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Valine. Additionally, fish meat was found to contain nine non-essential amino acids, which can be biosynthesized by the human body without external dietary substitution. TG2 exhibited the highest content of non-essential amino acids, constituting 11.08% of the fish weight, whereas TG3 demonstrated the lowest content at 9.15% of the fish weight. Nonetheless, it should be emphasized that these non-essential amino acids are inherently synthesized by the body, thereby allowing for the potential augmentation of nutrient absorption through the incorporation of diverse food sources into the diet.

It has been postulated that the inclusion of multiple animal protein sources in fish diets can enhance growth performance when juxtaposed with a singular protein source (Phonekhampheng, 2008). This phenomenon is presumably attributed to the equilibrium achieved in essential amino acid profiles derived from various sources.

The current study substantiates this notion; when substituting fishmeal with BSFL at rates of 10% and 30%, the total essential amino acid content increased by 7.4% and 6.8%, respectively, relative to the exclusive inclusion of protein from BSFL in the diet.

The findings underscore the high nutritional value of fish meat protein, given its comprehensive spectrum of essential and non-essential amino acids. It is noteworthy that the substitution of fishmeal with BSFL at varying proportions did not engender dissimilarities in amino acid compositions; however, variations were observed in amino acid content. Specifically, when the substitution exceeded or equaled 30% of fishmeal with BSFL, a pronounced reduction in amino acid content within the fish meat was evident.

CONCLUSION

This is the first study on the addition of non-defatted BSFL as a protein-rich and nutritional additive to replace fishmeal on the growth, development and quality of fish in Vietnam. The 10% - 30% substitution of fishmeal with BSFL in the diet of loach fish improved the weight growth (increased 6.27 g compared to 5.45 g without substitution). However, the length growth of fish with BSFL substitution was found to be significantly different with the control. The analysis results show that the correlation between length and weight is positive. However, the substitution at different ratios did not affect the survival rate and the feed conversion ratio of the fish. When rearing with a BSFL substitution at different concentrations, they all contained all the amino acids necessary for human and animal bodies. When replacing 10% of fishmeal with BSFL, the total amino acid content reached the highest value (11.08%).

ACKNOWLEDGEMENT

Not applicable.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Informed consent is not applicable. This study adhered to relevant national laws and guidelines. All procedures complied with Vietnam's Law on Veterinary Medicine (79/2015/QH13) and the Law on Animal Husbandry (32/2018/QH14).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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