



Effect of bioactive peptides obtained from *Tenebrio molitor* on growth, somatic indices, body composition, biochemical parameters and digestive enzyme activity of silver catfish

[Efeito de peptídeos bioativos, obtidos a partir de *Tenebrio molitor*, sobre crescimento, índices somáticos, composição corporal, parâmetros bioquímicos e atividade de enzimas digestivas de jundiás]

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Abstract: As aquaculture activity grows to meet population demand, farming systems intensify. Therefore, new research seeks to find nutritional additives that have a positive impact, reducing their breeding time and mortality. The present study addresses the effects of the inclusion of increasing levels of bioactive peptides obtained from *Tenebrio molitor* (BPTM) on the growth, somatic indices, body composition, circulating metabolites and digestive enzyme activity of juvenile silver catfish (*Rhamdia quelen*). Three isoproteic and isocaloric diets were formulated to contain levels of 0 %, 0.5 % or 1.0 % inclusion of BPTM. In a water recirculation system, 336 jundiá juveniles (initial average weight of 3.83 ± 0.69 g) were distributed in 12 experimental units (four replicates per treatment). Fish were fed the experimental diets to apparent satiety for six weeks. At the end of the trial, biometric measurements, blood samples and body tissue samples were collected. No significant differences were observed for final weight, total length, weight gain, condition factor, somatic digestive index, somatic hepatic index and intestinal quotient among the animals in the different treatments. Regarding body composition, there were differences in moisture and ash values (higher concentrations in fish in the 0.5 % and 1.0 % BPTM treatments, respectively). The activity of intestinal trypsin and chymotrypsin was not affected, as well as the plasma parameters evaluated. It is concluded that the inclusion of BPTM did not negatively affect the parameters evaluated, but further studies need to be carried out to elucidate an ideal level of peptide inclusion.

Keywords: pisciculture; functional food; animal growth; health promoter; *Rhamdia quelen*.

Resumo: Conforme a atividade aquícola cresce para atender a demanda populacional, os sistemas de cultivo se intensificam. Assim, novas pesquisas buscam encontrar aditivos nutricionais que reflitam positivamente, reduzindo seu tempo de criação e mortalidade. O presente estudo aborda os efeitos da inclusão de níveis crescentes de peptídeos bioativos obtidos a partir de *Tenebrio molitor* (PBTM) sobre o crescimento, índices somáticos, composição corporal, metabólitos circulantes e

atividade de enzimas digestivas de juvenis de jundiá (*Rhamdia quelen*). Três dietas isoprotéicas e isocalóricas foram formuladas para conter níveis de 0 %, 0,5 % ou 1,0 % de inclusão de PBTM. Em um sistema de recirculação de água foram distribuídos 336 juvenis de jundiá (peso médio inicial de $3,83 \pm 0,69$ g) em 12 unidades experimentais (quatro repetições por tratamento). Os peixes foram alimentados com as dietas experimentais até a saciedade aparente durante seis semanas. Ao final do ensaio, foi realizada biometria, coleta de sangue e de tecidos corporais. Não foram observadas diferenças significativas para o peso final, comprimento total, ganho de peso, fator de condição, índice digestivo somático, hepato somático e quociente intestinal, entre os animais dos diferentes tratamentos. Quanto à composição corporal, houve diferença nos valores de umidade e cinzas (maiores concentrações nos peixes dos tratamentos 0,5 % e 1,0 % de PBTM, respectivamente). A atividade de tripsina e quimotripsina intestinal não foi afetada, bem como os parâmetros plasmáticos avaliados. Conclui-se que a inclusão de PBTM não afetou negativamente os parâmetros avaliados, porém mais estudos precisam ser realizados para elucidar um nível ideal de inclusão de peptídeos.

Palavras-chave: piscicultura; alimento funcional; crescimento animal; promotor de saúde; *Rhamdia quelen*.

1. Introduction

Aquaculture is becoming increasingly important for generating income, employment, social development, and food and nutritional security worldwide. Current challenges in aquaculture involve the efficient use of natural resources to ensure healthy stocks and guarantee safe food supplies, alongside production practices that reduce environmental impacts and promote sustainable development ⁽¹⁾. In this sense, the search for improved economic indicators in aquaculture has stimulated research into nutritional strategies aimed at increasing disease resistance and reducing fish mortality during the rearing stages ^(2–4).

The expansion of aquaculture inevitably involves production intensification. Intensive fish farming can introduce predisposing factors that increase stress in various commercial species, ultimately causing immunosuppression and increasing susceptibility to diseases during the production cycle ^(5, 6). Therefore, there has been a notable increase in research aimed at developing novel functional additives, such as plant-derived bioactive compounds, hydrolysates, and peptides, which provide improve health, growth, immune responses, and disease resistance ^(7–11).

Insects, well-known for their high protein content and efficient feed conversion rates, represent a promising source of bioactive peptides ⁽¹²⁾. Bioactive peptides are small molecules formed by specific peptide bonds. Within their intact primary structure, peptides do not exhibit bioactivity, requiring enzymatic hydrolysis to release sequences that exhibit some type of bioactive function ⁽¹³⁾. Insect-derived peptides are recognized for their anti-inflammatory, antihypertensive, antidiabetic, antimicrobial, and enzyme-inhibiting properties, such as inhibiting lipase and angiotensin-converting enzyme ^(12,14).

The silver catfish (*Rhamdia quelen*) is an omnivorous species that has gained prominence in the aquaculture of native fishes in southern Brazil ⁽¹⁵⁾. It has highly desirable aquaculture characteristics, including the ability to survive at low temperatures (down to 3 °C), provided it is slowly adapted ⁽¹⁶⁾, efficient use of commercial diets, and broad adaptability to diverse farming conditions. Furthermore, its meat is highly appreciated by consumers for its favorable flavor and boneless fillets ⁽¹⁷⁾. However, when raised in intensive systems, stress factors may negatively affect its growth ^(18–20). Therefore, dietary supplementation with bioactive peptides may act as a nutritional

strategy to improve the growth performance and immune response of this species, while also providing insights for future research on other teleosts ^(2, 21, 22). Thus, this study aimed to evaluate the effects of dietary bioactive peptide supplementation on the growth, somatic indices, body nutrient and energy deposition, digestive enzyme activities, and plasma biochemical responses of juvenile silver catfish.

2. Material and methods

2.1 *Tenebrio molitor* production

T. molitor larvae were reared at the Animal Biodiversity Laboratory of the Federal University of Pampa, Uruguiana campus (Rio Grande do Sul, southernmost Brazil). The larvae were fed a commercial chicken feed composed of ground corn, corn gluten meal, soybean meal, and rice bran. This diet contained 19.96 ± 0.70 % crude protein, 3.83 ± 0.13 % lipids, 89.53 ± 1.16 % dry matter, and 10.28 ± 0.98 % mineral matter. The larvae were reared for 80 days until they reached a length of 2 cm.

2.2 Bioactive peptide preparation

The extraction protocol followed the method described by Villanova et al. ⁽²³⁾. *T. molitor* larvae were partially dried in an oven (55 °C for 48 h) and ground to obtain an insect meal. Subsequently, the meal was defatted via four consecutive washes with hexane (1:2 w/v). A sample of the defatted meal was homogenized and pre-incubated in distilled water (1:10 w/v) at 55 °C for 30 min. After pre-incubation, the enzyme Alcalase (0.04 %) was added, and the pH was adjusted to 7.0 using 0.1 N NaOH for enzymatic action. After an 8-h hydrolysis step at 55 °C, the enzyme was inactivated by heating the suspension at 95 °C for 15 min. The resulting mixture was cooled to 20–25 °C in an ice bath and centrifuged (8000 rpm for 20 min) to isolate insoluble materials from the soluble peptides.

2.3 Experimental diets

For the nutritional assay, three isoproteic (360 g Kg^{-1} of crude protein) and isocaloric (13.55 MJ Kg^{-1} of digestible energy) diets were formulated to meet the nutritional requirements of *R. quelen* ^(24–26). The diets were supplemented with increasing levels of bioactive peptides obtained from *T. molitor* larvae (BPTM), as follows: 0 % (control diet), 0.5 % BPTM, and 1.0 % BPTM (Table 1). Dietary ingredients were weighed and manually mixed until completely homogeneous. Water was then added to bring the mixture to a pelleting point. Immediately afterward, each experimental diet was pelleted in a meat grinder and kept in an oven at 55 °C for 24 h, packaged in labeled plastic bags, and stored in a freezer at -18 °C until fed to the animals.

Table 1. Formulation and proximate composition of the experimental diets (g Kg⁻¹).

Ingredients	Experimental diets		
	Control	0.5 % BPTM	1.0 % BPTM
Fish meal	670.0	670.0	670.0
Corn starch	159.8	159.8	159.8
Microcrystalline cellulose	60.0	55.0	50.0
Bioactive peptide	0.0	5.0	10.0
Linseed oil	30.0	30.0	30.0
Vitamins and minerals ¹	30.0	30.0	30.0
Iodized sodium chloride	5.0	5.0	5.0
Butylated hydroxytoluene	0.2	0.2	0.2
Inert ²	45.0	45.0	45.0
Total	1000.0	1000.0	1000.0
Proximate composition			
Crude protein ³	360.4	360.4	360.4
Digestible energy (MJ Kg ⁻¹) ⁴	13.55	13.55	13.55
Ether extract ³	103.9	103.9	103.9
Starch ³	159.8	159.8	159.8
Ash ³	157.1	157.1	157.1
Ratio DE:CP	89.82	89.82	89.82
Calcium ³	31.5	31.5	31.5
Phosphorus ³	16.1	16.1	16.1
Ratio Ca:P	1.96	1.96	1.96

¹Vitamin and mineral mixture (per kg of diet): Folic acid, 997.50 mg; Pantothenic acid, 9975.00 mg; Biotin, 159.60 mg; Cobalt, 39.90 mg; Copper, 2800.00 mg; Ethoxyquin, 24.78 g; Iron, 19.62 g; Iodine, 120.00 mg; Manganese, 5200.00 mg; Niacin, 19.95 g; Selenium, 119.70 mg; Zinc, 28.00 g; Vitamin A, 1995000 UI; Vitamin B1, 4987.50 mg; Vitamin B12, 5985.00 mg; Vitamin B2, 4987.50 g; Vitamin B6, 4987.50 mg; Vitamin C, 70.00 g; Vitamin D3, 198000.05 UI; Vitamin E, 19950.00 UI; Vitamin K, 997.50 mg. ²Sand; ³Calculated based on the analysis of the ingredients, and expressed in natural matter. ⁴Calculated Digestible Energy = ([crude protein x 23.61 MJ/Kg x 0,9] + [fat x 39.82 MJ/Kg x 0.85] + [soluble carbohydrates x 17.21 MJ/Kg x 0.50])⁽²⁷⁾.

2.4 Experimental trial

This study was approved by the institution's Ethics Committee on the Use of Animals (protocol no. 009/2021) and conducted at the Fisheries and Aquaculture Technology Center of the institution, over a six-week period. The feeding trial was conducted in a recirculating aquaculture system (polypropylene tanks 280-L working volume), a motor pump, and a biological filter. A total of 336 juvenile silver catfish (initial average weight 3.83 ± 0.69 g) were distributed into 12 experimental tanks (28 fish/tank). The study employed a completely randomized design consisting of three treatments with four replicates each. Fish were fed to apparent satiety twice daily (at 9:30 am and 4:30 pm) for six weeks. Tanks were siphoned daily at 8:30 am and 3:30 pm to remove feces.

Water quality parameters were monitored throughout the experiment. Temperature and dissolved oxygen were measured twice daily using a digital oximeter. The average observed temperatures were 18.70 ± 1.80 °C in the morning and 19.37 ± 1.43 °C in the afternoon, while dissolved oxygen concentrations were 6.07 ± 0.90 mg L⁻¹ in the morning and 5.81 ± 0.74 mg L⁻¹

in the afternoon. Additional water quality parameters were evaluated weekly, with overall means maintained within the following ranges: total alkalinity $235.83 \pm 29.40 \text{ mg L}^{-1} \text{ CaCO}_3$, total hardness $134.00 \pm 38.43 \text{ mg L}^{-1} \text{ CaCO}_3$, pH 7.70 ± 0.40 , total ammonia $0.71 \pm 0.74 \text{ mg L}^{-1}$, and nitrite $0.14 \pm 0.10 \text{ mg L}^{-1}$.

2.5 Data collection and performance parameters

At the end of the experimental period, fish were fasted for 18 h before the biometric evaluations. Two fish per experimental unit (eight per treatment) were sampled for blood collection via caudal vein puncture using heparinized syringes. Blood samples were centrifuged ($1000 \times g$ for 10 min) to obtain plasma for subsequent analyses. All remaining fish were then anesthetized with eugenol (clove oil) at 50 mg L^{-1} for biometry⁽²⁸⁾. Body weight (g) and total length (cm) were recorded and the following performance parameters were estimated:

- Condition factor: $\text{weight} / (\text{total length})^3 \times 100$;
- Weight gain (g): $\text{final weight} - \text{initial weight}$;
- Apparent feed conversion: $\text{feed consumed} / \text{weight gain}$;
- Survival (%): $(\text{final number of fish} / \text{initial number of fish}) \times 100$.

For analysis of somatic parameters, eight fish per treatment were euthanized via a eugenol overdose (500 mg L^{-1})⁽²⁹⁾ and used to determine the following parameters:

- Somatic digestive index (%): $(\text{weight of digestive tract} / \text{weight of whole fish}) \times 100$;
- Hepato-somatic index (%): $(\text{liver weight} / \text{weight of whole fish}) \times 100$;
- Intestinal quotient: $(\text{length of digestive tract} / \text{total length of fish}) \times 100$.

Immediately after collecting these parameters, the liver, stomach and intestine were stored in Eppendorf tubes and kept frozen (-18°C) for later analyses.

2.6 Body composition and nutrient deposition

To determine the proximate composition of whole fish and calculate nutrient deposition, eight animals from each treatment were euthanized via a eugenol overdose (500 mg L^{-1}) and used to determine the moisture, ash (muffle furnace at 550°C for 4 h), and crude protein contents (Micro-Kjeldahl method, using a 6.25 nitrogen-to-protein conversion factor) as outlined by the AOAC⁽³⁰⁾. Lipid content was evaluated using the cold extraction method, according to Bligh and Dyer⁽³¹⁾. An initial sample of 10 fish was collected to analyze the proximate composition of the whole fish and calculate deposition indices.

The body protein and fat deposit indices were calculated using Equations 3 and 4:

$$\text{Body protein deposition (g)} = [\text{FW} \times (\% \text{FBP} / 100)] - [\text{IW} \times (\% \text{IBP} / 100)] \quad (3)$$

$$\text{Body fat deposition (g)} = [\text{FW} \times (\% \text{FBF} / 100)] - [\text{IW} \times (\% \text{IBF} / 100)] \quad (4)$$

Where FW is the final weight, IW is the initial weight, IBP is the initial body protein, FBP is the final body protein, IBF is the initial body fat, and FBF is the final body fat.

2.7 Plasma analyses

In plasma, glucose (mg dL^{-1}), total cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), total protein (g dL^{-1}), and albumin (g dL^{-1}) levels were analyzed using commercial colorimetric kits (Bioclin®, Brazil). Globulin content (g dL^{-1}) was determined by difference: $(\text{total protein} - \text{albumin})$.

2.8 Digestive enzyme activity

The whole intestine was homogenized in a buffer solution containing 10 mM potassium phosphate and 20 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, in 50 % (v/v) glycerol.

A tissue-to-buffer ratio of 1:20 was utilized, and homogenization was performed using a Potter-Elvehjem homogenizer (Tecnal, Brazil). After homogenization, the samples were centrifuged at $1200 \times g$ for 10 min, and the supernatant was stored at -18°C to serve as an enzymatic source.

The activity of alkaline protease activity was determined according to Hummel⁽³²⁾. Trypsin activity was assayed using N α -p-Tosyl-L-arginine methyl ester hydrochloride (TAME) as the substrate. The intestinal homogenates were incubated for 2 min in 2 mL of Tris/CaCl₂ buffer (pH 8.1), and absorbance was measured at 247 nm using a spectrophotometer. Enzyme activity was expressed as $\mu\text{mol TAME min}^{-1} \text{ mg protein}^{-1}$.

Benzoyl-L-tyrosine ethyl ester (BTEE) was used as the substrate to determine chymotrypsin activity. The extracts were incubated for 2 min at 30°C in 2 mL of Tris/CaCl₂ buffer (pH 7.8), and absorbance was measured at 256 nm. Chymotrypsin activity was expressed as $\mu\text{mol BTEE min}^{-1} \text{ mg protein}^{-1}$. The soluble protein content of the samples, used to calculate specific enzyme activity, was determined according to the method of Bradford⁽³³⁾, using bovine serum albumin as the standard.

2.9 Statistical analyses

Data were initially tested for normality and subsequently subjected to a one-way analysis of variance. Treatment means were compared using Tukey's test at a 5 % significance level. All statistical analyses were conducted using SPSS version 21.0 software.

3. Results

At the end of the six-week experiment, no significant differences were observed in the performance variables or survival rate of the fish supplemented with bioactive peptides compared to the control treatment ($P > 0.05$) (Table 2). Similarly, the somatic indices did not differ among the fish supplemented with different levels of bioactive peptides.

Table 2. Performance parameters and somatic indices of silver catfish (*R. quelen*) supplemented with increasing levels of bioactive peptides.

Parameters	Experimental diets		
	Control	0.5 % BPTM	1.0 % BPTM
Final weight (g)	6.38 $\pm$ 2.11 ^a	6.16 $\pm$ 1.84 ^a	6.45 $\pm$ 2.47 ^a
Total length (cm)	8.95 $\pm$ 0.91 ^a	8.90 $\pm$ 0.82 ^a	8.96 $\pm$ 1.00 ^a
Condition factor	0.86 $\pm$ 0.12 ^a	0.85 $\pm$ 0.07 ^a	0.86 $\pm$ 0.09 ^a
Weight gain (g)	2.54 $\pm$ 0.28 ^a	2.34 $\pm$ 0.49 ^a	2.61 $\pm$ 0.15 ^a
Apparent feed conversion	2.61 $\pm$ 0.44 ^a	3.23 $\pm$ 0.48 ^a	2.99 $\pm$ 0.34 ^a
Survival (%)	99.00 $\pm$ 0.00 ^a	100.00 $\pm$ 0.00 ^a	100.00 $\pm$ 0.00 ^a
Intestinal quotient	1.27 $\pm$ 0.17 ^a	1.33 $\pm$ 0.23 ^a	1.40 $\pm$ 0.15 ^a
Hepatosomatic index (%)	2.03 $\pm$ 0.44 ^a	2.04 $\pm$ 0.50 ^a	2.05 $\pm$ 0.28 ^a
Digestive somatic index (%)	5.05 $\pm$ 1.40 ^a	5.90 $\pm$ 1.66 ^a	6.55 $\pm$ 2.11 ^a

Data are presented as mean $\pm$ standard deviation ($n = 4$). Different letters in the table rows represent a significant difference by Tukey's test ($P < 0.05$).

3.1 Body composition and nutrient deposition

At the end of the experimental period, no differences ($P > 0.05$) were observed in the crude protein and ether extract levels across all fish supplemented with increasing levels of bioactive peptides (Table 3). The silver catfish in the control group showed significantly lower water levels than 0.5 % BPTM and 1.0 % BPTM groups. Regarding mineral matter levels, the fish that received

the 1.0 % BPTM diet showed higher levels than the other groups. Body protein deposition remained unaltered among treatments, whereas body fat deposition was the highest in fish from the 1.0 % BPTM treatment, followed by the control and 0.5 % BPTM groups, respectively.

Table 3. Body Composition and nutrient deposition of silver catfish (*R. quelen*) supplemented with increasing levels of bioactive peptides.

Parameters	Experimental diets		
	Control	0.5 % BPTM	1.0 % BPTM
Moisture (%)	74.12±0.37 ^c	76.58±0.47 ^a	75.40±0.06 ^b
Crude protein (%)	15.50±0.54 ^a	15.71±1.39 ^a	16.22±1.01 ^a
Ether extract (%)	3.82±1.01 ^a	3.18±1.21 ^a	4.48±0.91 ^a
Ash (%)	3.20±0.13 ^b	2.77±0.23 ^b	3.92±0.16 ^a
Body deposition protein (g)	0.44±0.04 ^a	0.47±0.08 ^a	0.49±0.02 ^a
Body deposition fat (g)	0.11±0.01 ^b	0.06±0.01 ^c	0.15±0.00 ^a

Data are presented as mean ± standard deviation (n = 8). Different letters in the table rows represent a significant difference by Tukey's test ($P < 0.05$).

3.2 Plasma biochemical parameters

After six weeks of feeding the experimental diets, the silver catfish showed no significant differences ($P > 0.05$) in blood biochemical parameters (Table 4).

Table 4. Plasma biochemical parameters of silver catfish (*R. quelen*) supplemented with increasing levels of bioactive peptides.

Paramters	Experimental diets		
	Control	0.5 % BPTM	1.0 % BPTM
Glucose (mg dL ⁻¹)	48.00±10.80 ^a	55.16±13.98 ^a	50.75± 6.68 ^a
Total cholesterol (mg dL ⁻¹)	72.76±21.11 ^a	77.23±31.92 ^a	81.16±26.40 ^a
Triglycerides (mg dL ⁻¹)	51.90±16.76 ^a	59.46±25.09 ^a	72.05±20.02 ^a
Albumin (g dL ⁻¹)	0.44±0.16 ^a	0.74±0.33 ^a	0.71±0.11 ^a
Total protein (g dL ⁻¹)	3.16±1.07 ^a	3.14±1.01 ^a	3.03±0.26 ^a
Globulins (g dL ⁻¹)	2.70±0.84 ^a	2.22± 0.40 ^a	2.24± 0.40 ^a

Data are presented as mean ± standard deviation (n = 8). Different letters in the table rows represent a significant difference by Tukey's test ($P < 0.05$).

3.3 Digestive enzyme activity

After receiving the experimental diets for six weeks, the silver catfish showed no significant differences ($P > 0.05$) in trypsin or chymotrypsin activity (Figure 1).

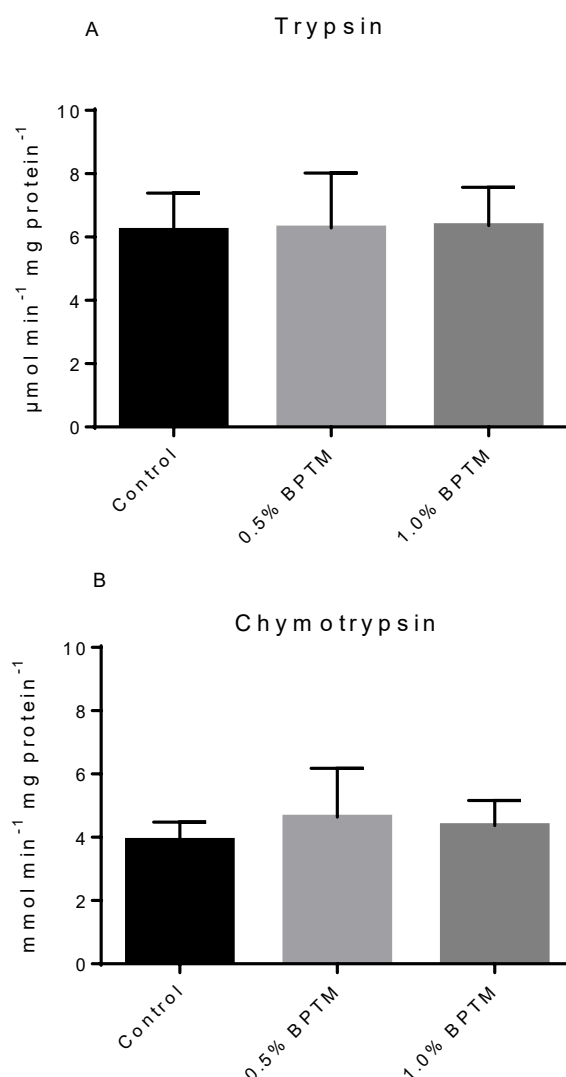


Figure 1. Digestive enzyme activity of silver catfish (*R. quelen*) supplemented with increasing levels of bioactive peptides. Data are presented as mean $\pm$ standard deviation ($n = 8$). No differences were observed using Tukey's test ($P < 0.05$).

4. Discussion

Considerable efforts have been made to reduce antibiotic use in aquaculture, leading to the application of natural supplements aimed at promoting growth and conferring health benefits. In this sense, different sources of bioactive peptides have been evaluated in the nutrition of different fish species ^(34–37). Nevertheless, studies investigating insect-derived additives with growth-promoting potential remain scarce.

In this study, incorporating increasing levels of bioactive peptides obtained from *T. molitor* larvae did not affect growth parameters. Similarly, the survival and somatic indices of silver catfish fed the different diets did not show any influence by the peptide inclusion levels. It is not yet well understood, although evidence suggests that the growth parameters are not influenced by peptide inclusion because the dosage used was below the ideal. According to Sarker et al. ⁽³⁸⁾, peptide

bioactivity and bioavailability are influenced by several factors, such as molecular weight, amino acid composition, and sequence, which may be directly related to their method of obtainment. To ensure functionality, these peptides must remain unaltered and readily available for intestinal absorption. Because research on extracting peptides from *T. molitor* larvae is nascent, future studies should be conducted to elucidate ways to enhance the bioactivity of these compounds without impairing their absorption or growth-promoting efficacy, thus considering their wider application in aquaculture.

Evaluating fish body composition is an important tool for assessing the efficiency of nutrient transfer from the diet to the animal ⁽³⁹⁾. In this study, including bioactive peptides altered body composition parameters. Fish receiving 0.5 % BPTM in their diet showed significantly higher body water content than those in other treatments, whereas mineral content was significantly higher in catfish supplemented with 1.0 % BPTM. Water is the main constituent of fish (accounting for 60–85 % of the edible portion); it is structurally partitioned into constitutional water bound to proteins and carbohydrates (15–25 %), and free water present in muscle and other tissues. Regarding minerals, most metallic atoms are present in fish muscle. Elements such as sodium, potassium, calcium, and chlorine are found in the inorganic state, while phosphorus, sulfur, iodine, iron, copper, and cobalt are typically bound to proteins, fats, and carbohydrates ⁽³⁹⁾.

Water and mineral matter are negatively correlated, since water is expressed by the sample's weight loss after the drying process, and the volume of mineral matter is given by the incineration of organic matter ⁽⁴⁰⁾. Notably, fish fed the 0.5 % BPTM diet showed a higher carcass water content. This may indicate that the 0 and 0.5 % treatments had lower mineral solubility compared to the 1.0 % BPTM treatment, which yielded greater mineral matter. Andriamialinirina et al. ⁽⁴¹⁾ observed that diets supplemented with 1 % yeast hydrolysate reduced mineral matter content in juvenile *Nile tilapia* (*Oreochromis niloticus*) supplemented with 1 % and higher mineral matter content in the 0 and 3 % inclusion levels increased it. Furthermore, supplementing pompano (*Trachinotus blochii*) diets with up to 20 % tuna viscera hydrolysate did not reflect differences in body water or mineral matter ⁽⁴²⁾. Similarly, replacing fishmeal with 10 % *T. molitor* hydrolysate in diets for sea trout (*Salmo trutta* m. *trutta*) did not show differences in body water or mineral matter values compared to other treatments ⁽⁴³⁾.

According to Li et al. ⁽⁴⁴⁾, blood biochemical parameters and digestive enzyme activities are reliable indicators of fish nutritional and health status. In our study, no differences in blood biochemical parameters were observed among treatments, indicating that these values align to those reported by Adorian et al. ⁽⁴⁵⁾: glucose 73–74 (mg dL⁻¹), cholesterol 75–93 (mg dL⁻¹), and total protein 2–3.05 (g dL⁻¹). In this way, there was no impairment of the blood parameters evaluated, the same occurred with diets containing up to 20 % soy hydrolysates for silver catfish in which no treatment obtained differences in blood parameters ⁽⁴⁶⁾. These findings demonstrate that bioactive peptides derived from *T. molitor*, when included in the diet at levels up to 1.0 %, do not negatively affect fish health.

Naveed et al. ⁽⁴⁷⁾ noted that the nature of the food and the feeding habits of fish directly influence their digestive enzyme activity and nutrient assimilation. Therefore, the intestine is considered an effective indicator of animal performance and health, due to its function in immune defense, digestion, and nutrient absorption. No significant differences were observed in the digestive enzyme activity of fish fed increasing levels of bioactive peptides, corroborating findings wherein the inclusion of 20 % *T. molitor* hydrolysate in sea trout diets resulted in no differences in trypsin activity ⁽⁴⁸⁾. In a study testing 25 and 50 % fish muscle hydrolysate, squid mantle, and

soybean meal in diets for giant snapper larvae (*Lates calcarifer*), Srichanun et al.⁽⁴⁹⁾ observed significant differences in chymotrypsin activity with increasing levels of alcalase-derived squid mantle hydrolysate; however, trypsin activity remained unchanged relative to the control group.

These results are promising, demonstrating that digestive enzyme activity was not negatively influenced and that nutrient digestion and assimilation process of nutrients followed a similar pattern to the group that received a control diet. In diets containing insects or insect derivatives, digestive enzyme activity may decrease due to the presence of chitin from the insects⁽⁴⁶⁾. Because this reduction was absent in this research, it can be inferred that the peptide extraction process effectively reduced or eliminated insect-derived antinutritional factors, including chitin.

5. Conclusion

The inclusion of *T. molitor*-derived bioactive peptides at levels up to 1.0 % in diets was well accepted by the juvenile silver catfish, yielding no adverse effects on growth performance, somatic indices, digestive enzyme activity, or plasma biochemistry. These establish a benchmark for future research seeking to elucidate the ideal levels of peptide inclusion in silver catfish diets.

Conflict of interest statement

The authors declare no competing interests.

Data availability statement

The data will be provided upon request to the corresponding author.

Author contributions

Conceptualization: Ferrigolo, F. R. G.; Pretto, A.; Formal analysis: Ferrigolo, F. R. G.; Pretto, A.; Rodrigues, A. T.; Funding acquisition: Ferrigolo, F. R. G.; Pretto, A.; Methodology: Penchel, E. M.; Serra, S. D. S.; Martins, G. B.; Rodrigues, A. T.; Ferrigolo, F. R. G.; Pretto, A.; Supervision: Ferrigolo, F. R. G.; Writing (original draft): Penchel, E. M.; Rodrigues, A. T.; Martins, G. B.; Pretto, A.; Writing (revision and editing): Ferrigolo, F. R. G.; Pretto, A.; Martins, G. B.; Rodrigues, A. T.; Penchel, E. M.; Serra, S. D. S.

Generative AI use statement

The authors did not use generative Artificial Intelligence tools or technologies in the creation or editing of any part of this manuscript.

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