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EFFECTS OF A NUTRITION SOURCE OF HYDROLYZED PROTEIN FROM WHITELEG SHRIMP HEAD (*Litopenaeus vannamei*) IN A PROCESS OF *Trichogaster pectoralis's* NURTURING

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Abstract. The study explored the use of small peptides and oligopeptides derived from hydrolyzed whiteleg shrimp head protein (*Litopenaeus vannamei*) to nurture *Trichogaster pectoralis* (Pearl Gourami). These peptide fragments were analyzed for amino nitrogen content and molecular weight using the OPA and SDS-PAGE methods. Two types of feed were tested: control feed (CF) and processed feed (PF), both containing fish meal, to evaluate the growth of Pearl Gourami. The results showed that both feeds led to low concentrations of dissolved oxygen (DO) (4.48 to 6.49 mg/L), chemical oxygen demand (COD) (16.09 to 24.98 mg/L), and biological oxygen demand (BOD) (16.62 to 22.61 mg/L), which were within the acceptable limits set by national aquaculture standards. Additionally, the survival rate of the fish remained highly, ranging from 94.8% to 99% after 60 days. In terms of growth performance, fish fed with PF exhibited weight gain (WG) of 7.00±0.76 g/fish, daily weight gain (DWG) of 0.117±0.014 g/fish/day, specific growth rate (SGR) of 0.672±0.065 %/g/fish, feed intake (FI) of 0.467±0.002 g/fish/day, and a feed conversion ratio (FCR) of 4.16±0.314. The digestibility coefficients of dry matter (ADC_{DM}), total protein (ADC_{pro}), and total lipid (ADC_{Lip}) for fish fed with PF were 66.2%±1.92, 90.4%±0.468, and 88.3%±0.576, respectively. Furthermore, the average meat weight of Pearl Gourami was 2.08±0.221 grams.

Keywords: digestibility, growth, oligopeptide, processed feed, small peptides, *Trichogaster pectoralis*

1 Introduction

Trichogaster pectoralis (Regan, 1910) has gradually become the main species of fish culture in several provinces in the Mekong Delta besides polyculture with other fish species [1]. Despite the increasing demand of *Trichogaster pectoralis* (Pearl Gourami), the yield and profit from this fish are still not stable as those depend on the feed source and the culturing season. While, whiteleg shrimp is a key export processed seafood product of Vietnam with high and stable economic efficiency. According to the Directorate of Fisheries in 2020, Vietnam's *Litopenaeus vannamei* (Boone, 1931) production was twice times higher than the production of black tiger shrimp in recent years. The increasing production of shrimp means that a large amount of by-products is generated every year. These by-products mostly include shrimp heads and shells, which are considered as a rich source of protein and contain many biological substances such as vitamins, chitin, carotenoids, etc.., [2] and are suitable for use as animal feed. In addition, there has been a lot of research on the application of protease from plants in many fields such as food processing, medicine and processing industrial by-products in recent years [3]. Bromelain (from pineapple) is one of the most widely used thanks to its hydrolysis activity of protein. The stems, leaves, and peelsof pineapple are often discharged into the environment because they are non-valuable for consumption [4]; however, they are a potential source of bromelain to hydrolyze protein sources into valuable peptides products [5].

Generally, in aquaculture, the feed always accounts for the largest cost because farmers often do not have the initiative in producing feed. Therefore, this study is carried out with the desire to create a new feed source for Pearl Gourami from inexpensive by-products that are easily to find on the market as well as investigate the effectiveness of using the processed feed for nurturing Pearl Gourami.

2 Materials and methods

2.1 Materials

The crude bromelain solution was extracted from pineapple peels belonging to the Queen pineapple variety (*Ananas comosus*). Whiteleg shrimp heads and shells (*Litopenaeus vannamei*) were purchased at Co Chien Seafood Joint Stock Company. The samples were washed, then drained and ground to a size of 2 to 3 mm and Pearl Gourami were purchased at Tu Dai hatchery (Thot Not District - Can Tho City).

2.2 Preparation for raising fish

Six tanks were designed two treatments and three replicates for aquaculture of *Trichogaster pectoralis* and were prepared with approximately 0.45 m^3 of water and stocked with 32 fish with size of 9 to 10 cm per individual (around 10 -12 gram/fish). The fish were fed from 2 to 3% (w/w) of the total weight of fish at 8 am and 16 pm every day. The water in tanks was replaced by approximately 30% every 15 days.

2.3 Preparation of feed

Shrimp heads was hydrolyzed by crude bromelain following the procedure of Vo Van Song Toan et al. [6] at 50 °C in 3 hours. The OPA method was used to determinate amino nitrogen content and the SDS-PAGE method was used to evaluate the appearance of fragments of proteins.

Feed for nurturing of Pearl Gourami were produced following of Tran Thi Thanh Hien and Nguyen Anh Tuan's the method [7]. Two treatments including of control feed (CF) and processed feed (PF) were fed as meal contained protein of shrimp head and hydrolyzed protein of shrimp head with the total protein content of feed around 25%. Both two types were mixed with 1% (w/w) Cr_2O_3 for calculating digestibility of fish. Total protein, total lipid, ash [8], amino nirogen [9] were the criteria for evaluating the nutritional of feed.

2.4 Evaluation of water quality

The water samples were collected randomly on the surface of the tanks every 15 days before changing new water for evaluation of concentrations of dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) following methods of APHA [10].

2.5 Evaluation of Pearl Gourami' growth

Criteria for evaluation of Pearl Gourami's growth including of survival rate (SR, %), weight gain (WG, g/fish), daily weight gain (DWG, g/fish/day), specific growth rate (SGR, %/fish/day), feed consumption (FC, g/fish) and feed conversion rate (FCR), protein efficiency ratio (PER, %) was calculated according to Nguyen Thi Linh Dan et al. [11] at the end of the nurturing period (after 60 days).

2.6 Determination of digestibility coefficient and nutritional compositions

Trichogaster pectoralis' feces were collected by siphoning after 24 hours of a meal. Chemical contents in feces such as dry matter, total protein and total lipid were analyzed according to AOAC [8]. Apparently digestibility coefficient (ADC) was calculated according to Glencross et al. [12] besides of ingredient nutrition of Pearl Gourami's meat.

2.7 Statistical analysis

All experiments were designed in a randomized design with three replicates for each treatment. The data were processed by Microsoft Excel 2016. The mean, standard deviation, standard error of data was analyzed by the Minitab 16. Comparison between treatment means was performed by one-way analysis of variance (ANOVA) and $p < 0.05$ was significantly different.

3 Results and discussion

3.1 Hydrolyzed protein solution and nutritional compositions of feed

Hydrolyzed protein solution

Table 1 shows that dry matter content from hydrolyzed protein of shrimp head reached 11.9% compared to 75.0% in shrimp head. In contrast, amino nirogen content from a hydrolyzed protein solution of shrimp head was 93.3mgN/g compared to 20.5 mgN/g amino nirogen content of shrimp head. In addition, a hydrolysis process of protein of shrimp heads produced small fragments including of small peptides and oligopeptides used as a supplement nutritional source of fish feed.

Besides, the results of the SDS-PAGE method showed that the protein extracted from shrimp heads had molecular weight ranging 37 to over116 kDa (fig. 1) and mostly hydrolyzed by bromelain into small fragments of protein with small molecular weight ranging from 24 to 16.5 and below 14 kDa. This result was consistent to the study of Gómez et al. [13], hydrolysis of shrimp by-products also gave similar results by the SDS-PAGE method with molecular weight of fragments around 7 to 37 kDa.

Table 1. Compositions of shrimp head

Material	**Dry matter** (%)	**Amino nirogen content** (mgN/g)
Shrimp head	75.0 ± 1.79	20.5 ± 0.206
Hydrolyzed protein of shrimp head	11.9 ± 0.081	93.3 ± 1.82

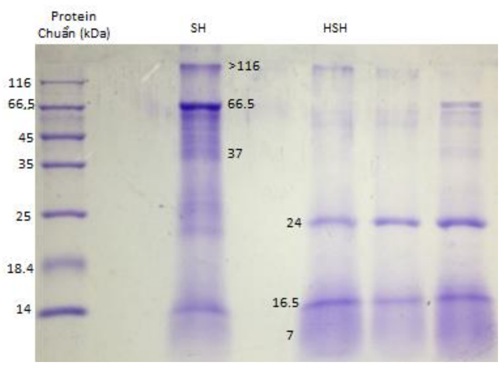

Fig. 1. Molecular weight of protein from whiteleg shrimp head

(*Note:* SH: Shrimp head; HSH: Hydrolyzed shrimp head)

Feed compositions

Table 2 illustrates that, a dry matter of the two feeds was around 91%; three nutritional components of the PF contained 26.9%, 19.3% and 7.81% of protein, total lipid and ash proportions and higher than that of the CF which had only 23.6%, 15.3% and 6.23%, respectively. In addition, the small peptides and oligopeptides content of PF (13.2 mgN/g) contained a greater proportion of amino nitrogen compared to the components in CF (6.05 mgN/g). Besides, the content of total protein, total lipid and ash in the PF was still slightly higher than in the CF. Moreover, shrimp are classified as crustaceans, the composition of the shrimp head also contains high levels of minerals such as copper, iron, zinc, calcium [2] contributing to increasing the nutrient content of the feed for fish growth.

3.2 Effects of feed on water quality of aquaculture

Table 3 shows that the dissolved oxygen (DO) concentration of the water had a decreasing trend and being highest in the tanks at the beginning of the experiment and reaching its lowest level in the tanks fed with PF after 60 days (4.48 mg/L). At the first water change cycle, the dissolved oxygen content in the tanks did not differ between the two feeds used (6.38 mg/L and 5.92 mg/L). However, from day 30th to day 60th, the DO content in the treatment PF were lower than that in the tanks using the CF.

Table 2. Nutritional compositions of feed

Feed type	Dry matter (%)	Total protein (%)	Amino nitrogen (mg N/g)	Total lipid (%)	Ash (%)
CF	91.5 ± 2.39	23.6 ± 0.72	6.05 ± 0.058	15.3 ± 2.00	6.23 ± 0.187
PF	91.1 ± 1.18	26.9 ± 1.72	13.2 ± 0.071	19.3 ± 2.98	7.81 ± 0.332

Note: Data present in the table are mean ± stdev. CF: Control feed; PF: Processed feed

Table 3. Parameter of dissolved oxygen demand

Feed type	Dissolved oxygen concentration (mg/L)				
	Day 1th	**Day 15th**	**Day 30th**	**Day 45th**	**Day 60th**
CF	$11.84^a \pm 0.15$	$6.38^a \pm 1.37$	$6.81^a \pm 0.62$	$6.93^a \pm 0.42$	$6.49^a \pm 0.062$
PF	$11.63^a \pm 0.13$	$5.92^a \pm 0.61$	$4.84^b \pm 0.72$	$5.07^b \pm 0.71$	$4.48^b \pm 0.655$
CV (%)	6.4	10.2	12.0	10.02	7.79

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at $P < 0.05$. CF: Control feed; PF: Processed feed

Chemical Oxygen Demand (COD) concentration in these tanks of two treatments had an increasing trend over time when fish were fed with 2 types of feed. COD had the lowest values in all tanks before feeding and this index was highest in the tanks fed with PF at day 45 (24.98 mg/L). Besides, the COD index of the tanks used PF on days 30^{th}, 45^{th} and 60^{th} were also significantly higher 1.4, 1.6 and 1.6 times than the others used CF (Table 4).

There was a gradual increase in the Biochemical oxygen demand (BOD) concentration when they were fed both types of CF and PF from 0.96 to 16.62 (table 5) and 1.01 to 22.61 (mg/L). In period of 30^{th}, 45^{th} and 60^{th} day, the BOD concentration of water in the tanks fed with PF was high such as 20.48, 21.55, 22.61 mg/L compared to 16.75, 14.19 and 16.62 mg/L from the BOD of water in tanks fed with the CF (Table 5).

According to the National Technical Regulations QCVN 01-80:2011/BNNPTNT, all tanks feeding with 2 types of feed had DO, BOD, COD concentrations within the allowable thresholds of national standards, suitable for the growth of Pearl Gourami.

In a case of *Trichogaster pectoralis*, the DO results ranged above 3 mg/L, which support good growth and reproduction of the species [14]. In addition, this result also showed that these values have a relationship with each other. When the DO concentration decreased, the COD and BOD concentrations increased due to the oxidation and respiration processes of living organisms.

Table 4. Parameter of chemical oxygen demand

Feed type	**Chemical oxygen demand** (mg/L)				
	Day 1^{th}	Day 15^{th}	Day 30^{th}	Day 45^{th}	Day 60^{th}
CF	$3.20^{a} \pm 0.153$	$12.62^{a} \pm 1.32$	$16.0^{b} \pm 0.53$	$14.76^{b} \pm 0.56$	$16.09^{b} \pm 0.812$
PF	$3.47^{a} \pm 0.100$	$19.38^{a} \pm 4.04$	$22.31^{a} \pm 2.83$	$24.0^{a} \pm 2.08$	$24.98^{a} \pm 1.82$
CV (%)	7.2	14.3	8.00	6.22	6.17

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at p<0.05. CF: Control feed; PF: Processed feed

Table 5. Parameters of biochemical oxygen demand

Feed type	**Biochemical oxygen demand** (mg/L)				
	Day 1^{th}	Day 15^{th}	Day 30^{th}	Day 45^{th}	Day 60^{th}
CF	$0.96^{a} \pm 0.208$	$10.19^{a} \pm 0.40$	$16.75^{b} \pm 0.56$	$14.19^{b} \pm 1.07$	$16.62^{b} \pm 0.73$
PF	$1.01^{a} \pm 0.100$	$11.84^{a} \pm 1.58$	$20.48^{a} \pm 0.85$	$21.55^{a} \pm 1.54$	$22.61^{a} \pm 1.40$
CV (%)	5.4	8.63	3.75	7.33	5.30

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at p<0.05. CF: Control feed; PF: Processed feed

Similarities was found with studies of Pham Quoc Nguyen et al. [15] and Bui Thi Nga [16], showed that in ponds of culturing catfish in Can Tho city, DO concentration decreased after the culture season along with a strong increase of COD and BOD concentrations.

The increase in COD and BOD concentrations was due to the accumulation of organic and inorganic substances from uneaten feed and fish feces which contains high levels of protein and lipid that are easily oxidized when exposed to the environment and decomposed by microorganisms [17]. Besides, the accumulation of potentially oxidized substances in the feces and inorganic compounds which were eliminated by the digestive process will also affect the COD and BOD in water. This was mentioned in the study of Timmons and Lorsodo [18] that if the solid waste is not treated but still remains in the tank water, it will lead to an increase in COD, BOD due to the activity of microorganisms. Organisms take place stronger to decompose excess substances, thereby making the DO concentration gradually decrease because microorganisms will compete for oxygen sources with fish, leading to fish being easily stressed and adversely affecting growth [19].

3.3 Evaluation of the effect of feeds on the nurturing of *Trichogaster pectoralis* (Regan, 1910)

Growth rate

Table 6 shows that *Trichogaster pectoralis* (Pearl Gourami) which were fed with the PF had a significantly superior growth (7 g/fish) in weight compared to 3.99 (g/fish) of the fish groups fed with the CF. Fish fed with the PF had an average WG and average DWG about 1.75 times higher than that of fish fed with the CF, and SGR was about 1.6 times higher than that of the other group.

The addition of small peptides and oligopeptide from hydrolyzed protein of whiteleg shrimp head into the PF gave a positive effect on the growth of fish. The PF helped to add flavor to the feed and also provided a soluble oligopeptide source that promoted the growth of fish. There were also significant differences in growth, survival and uniformity of *Trichogaster pectoralis*

Table 6. Weight growth of *Trichogaster pectoralis*

Treatment	**WG** (g/fish)	**DWG** (g/fish/day)	**SGR** (%/fish/day)
CF	$3.99^{b} \pm 0.82$	$0.067^{b} \pm 0.013$	$0.416^{b} \pm 0.070$
PF	$7.00^{a} \pm 0.76$	$0.117^{a} \pm 0.014$	$0.672^{a} \pm 0.065$
CV (%)	11.7	11.8	9.60

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at $p<0.05$. CF: Control feed; PF: Processed feed WG: Weight gain; DWG: Daily weight gain; SGR: Specific growth rate.

(Pearl Gourami) from 3 different fingerling sources in a study by Nguyen Hoang Thanh et al. [14] while they were fed with the same diet. This proves that the growth of fish depends not only on the quality of the feed, but also on the fish species and the source of fingerling.

Feed efficiency

A survival rate of *Trichogaster pectoralis* (Pearl Gourami) between the two groups the CF and the PF was 94.8 and 99% (Table 7). There was not statistically different but fish fed by the PF show higher survival rate than other group. Regarding efficiency of feed intake (FI), there was a significant difference between the two groups. Specifically, each day the fish group that used PF ate 0.173 g more than the group of fish that used the CF. Regarding the FCR, there was a difference when the fish ate the PF only needed 4.16 g of feed to convert to 1 g of weight, compared with 5.94 g of feed for the other group. In terms of PER, the fish group fed with the PF was higher than the fish group fed with the CF, namely 0.896 versus 0.726.

This result was similar to the study of Nguyen Thi Linh Dan et al. [20] when using a diet to replace fishmeal with soybean meal on bronze feather back fish, which also showed that the fish ate more when soybeans make up a lower percentage of the diet. This demonstrates that the addition of a hydrolyzed solution of whiteleg shrimp head positively impacts fish growth, as animal protein sources are more efficient than plant-based proteins [21]. Another study on African catfish [22] used shrimp head and shell by-products to replace soybean meal in the diet also gave similar results. Especially the diets containing shrimp shell and head which were treated in an acidic environment gave the highest PER and FCR results among the treatments. This proves that the protein source in the feed can be hydrolyzed into oligopeptides with small molecular weight under the influence of acid, helping fish absorb nutrients better, leading to better growth rate. Thereby, it can be seen that the potential of applying enzymatic hydrolyzed shrimp head by-products in creating animal feed is possible due to the same mechanism.

Table 7. Feed efficiency of *Trichogaster pectoralis*

Type feed	**SR** (%)	**FI** (g feed/fish/day)	**FCR**	**PER**
CF	$94.8^a \pm 1.80$	$0.294^b \pm 0.003$	$5.94^b \pm 0.952$	$0.726^a \pm 0.111$
PF	$99.0^a \pm 6.50$	$0.467^a \pm 0.002$	$4.16^a \pm 0.314$	$0.896^a \pm 0.066$
CV (%)	4.34	0.625	11.8	11.3

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at P<0,05. CF: Control feed; PF: Processed feed; SR: Survival rate; FI: Feed intake; FCR: Feed conversion ratio; PER: Protein efficiency ratio.

3.4 Determination of apparent digestibility coefficient

Table 8 shows that there was a significant difference between the two types of feed used for snakeskin gourami when the ADC of the PF had higher values than that of the CF. ADC of dry matter in the CF was about 8% lower than the PF. Similarly, ADC of total protein and total lipid of the PF was about 7% higher than that of the CF.

These results were similar to studies on the replacement of soybean meal in aquaculture feed. Research by Tran Thi Be et al. [23] showed that the ADC of the feed containing completely soybean meal on goby fish was only from 56.2 – 65.1%, and the ADC of total protein and total lipid of diets supplemented with fishmeal (an animal protein source similar to shrimp heads) gave high results, at 90.6% and 87.2%, respectively. A study by Ibrahim et al. [22] found that the ADC of soybean meal replacement with processed shrimp by-products was over 80%. Research by Rahman et al. [24] on flounder showed high digestibility results for diets using different fishmeal sources, with ADC values ranging from 69% to 84% for dry matter, 87 - 95% for total protein and 83 - 96% for total lipids.

Animal protein sources often have a more attractive taste than vegetable protein, thus attracting and stimulating fish to eat more, and at the same time, protein hydrolysis will create oligopeptides and free amino acids that are more easily absorbed by the digestive system of animal [25]. Therefore, the addition of oligopeptide solution from hydrolyzed protein of shrimp head increased the nutrient absorption of *Trichogaster pectoralis*. In addition, fish and other aquatic animals are cold-blooded animals, the body temperature changes depending on the temperature of the environment, leading to the enzyme system in the fish's body, especially the digestive tract enzyme system, will be affected because enzymes only work best in a certain temperature range. Therefore, oligopeptides and free amino acids are easier for fish to absorb compared to un-hydrolyzed protein sources.

Table 8. Apparent Digestibility Coefficients of *Trichogaster pectoralis*

Treatment	ADC_{DM} (%)	ADC_{Pro}(%)	ADC_{Lip}(%)
CF	$58.7^{b} \pm 1.14$	$83.7^{b} \pm 0.398$	$81.1^{b} \pm 0.485$
PF	$66.2^{a} \pm 1.92$	$90.4^{a} \pm 0.468$	$88.3^{a} \pm 0.576$
CV (%)	2.42	0.500	0.625

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at p<0.05. CF: Control feed; PF: Processed feed; DM: Dry matter; Pro: Total protein; Lip: Total lipid, ADC: Apparent digestibility coefficients.

3.5 The effects of feeds on the nutritional compositions of *Trichogaster pectoralis* (Regan, 1910)

The proportion of dry matter, protein and lipid content in the meat of *Trichogaster pectoralis* (Pearl Gourami) between two type of feed as the PF and the CF ranged from 23.8 to 23.9, 73.8 to 74.0 and 25.5 to 26.1% respectively, with no significant differences (Table 9). A water content of *Trichogaster pectoralis'* meat was high (around 76%) because they were entering the spawning period [1].

According to Murray et al. [26], the water content of fish in the reproductive stage increased along with the protein content decrease. Comparing this with the overall protein content of fish meat from 57.36 to 75.92% [26], the differences were quite high. This is somewhat dissimilar to the study on dried *Trichogaster pectoralis* (Pearl Gourami) meat with the results that the protein content of dried fish meat only ranges from 55 - 69%. Besides, the protein content of *Trichogaster pectoralis*' meat reached 73.8% and 74% for the fish consumed the CF and the PF. The lipid content of *Trichogaster pectoralis*' meat was around 25 - 26%. According to Mark [27], a removement of the fish skin was able to reduce lipid content in the skin. However, meat weight of *Trichogaster pectoralis* used the PF was 2.08 ± 0.221 g and significantly different compared to the others using the CF containing only 1.30 ± 0.170 g of *Trichogaster pectoralis*' meat, respectively.

Table 9. Nutritional compositions of *Trichogaster pectoralis'* meat

Treatment	Dry matter (%)	Crude protein (%)	Lipid (%)	Meat weight (g)
CF	$23.8^{a} \pm 2.25$	$73.8^{a} \pm 2.13$	$25.5^{a} \pm 0.278$	$1.30^{b} \pm 0.170$
PF	$23.9^{a} \pm 0.735$	$74.0^{a} \pm 0.674$	$26.1^{a} \pm 0.324$	$2.08^{a} \pm 0.221$
CV (%)	6.26	6.26	1.17	11.6

Note: Data present in the table are mean ± stdev. Means in the same column that share a letter are not significantly different at p<0.05. CF: Control feed; PF: Processed feed; Meat weight, protein weight and lipid weight were calculated based on dry matter.

4 Conclusions

This study indicated that processed feed (PF) supplemented with small peptides and oligopeptides from hydrolyzed protein of whiteleg shrimp head had a positive effect on the growth and digestibility coefficients of the *Trichogaster pectoralis* (Pearl Gourami) from 7 to 15 weeks of age. The results not only showed the water quality of culture water complied an allowable threshold of national standards for fishing but also improved growth of *Trichogaster* *pectoralis* to 67,2% of SGR. Furthermore, *Trichogaster pectoralis* also consumed and metabolized the PF effectively leading to high growth and survival rate.

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