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EVALUATION OF POLYUNSATURATED FATTY ACIDS AND β-GLUCAN CONTAINING DIET ON GROWTH PERFORMANCE AND CONDITION FACTOR OF PABDAH CATFISH, *Ompok pabda* (HAMILTON, 1822)

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ABSTRACT

A nutritionally balanced diet and selection of appropriate species are important for aquaculture. The present study was conducted to evaluate the effects of polyunsaturated fatty acids (PUFAs) and β-glucan containing diet (PBG) on growth performance, feed utilization, length-weight relationship, and condition factor of Pabdah catfish, *Ompok pabda*. In this study, squid extracted phospholipid and mushroom powder were used as the source of PUFAs and β-glucan, respectively, and formulated two isonitrogenous diets such as basal or control (CON) diet and PBG diet with maintaining 30% protein levels. Optimum physicochemical parameters of water such as dissolved oxygen (DO), temperature, and pH were maintained at 6.7 ± 0.5, 26.5 ± 2°C, and 7.4 ± 0.2, respectively in each cistern during the study period. The results showed that fish fed with the PBG diet had significantly ($P<0.01$) higher final body weight, final length gain, food conversion ratio (FCR), specific growth rate (SGR), food conversion efficiency (%), hepato-somatic index (HSI), kidney index (KI), and viscerosomatic index (VSI) than fish fed with the CON diet. The coefficient of determination revealed a significant positive relationship ($R^2 = 0.956$) between the treatment group's length and weight. The PBG diet had a significant ($P<0.05$) influence on the length-weight relationship and relative condition factor (K) of *O. pabda*. The current study demonstrates that the experimental diet improves growth performance, feed utilization, length-weight relationship, and condition factor of *O. pabda*.

KEYWORDS: feed utilization; hepato-somatic index; length-weight relationship; specific growth rate; nutrition

INTRODUCTION

The fish consumption rate is growing in the world with the increasing rate of human population (FAO, 2020). Fish and fish-related products are important sources of animal protein for humans globally (World Health Organization, 2004). However, feeding fish has become one of the critical management practices today, because the sustainability of fish production depends on proper feeding and farm management. Fish nutrition is one of the most important factors influencing the growth performance of silver barb (Jahan *et al.,* 2020). Lack of aquaculture knowledge makes the farmers oblivious of nutrient availability in the raw materials and the nutritional requirement of the Indian carp (Adikari *et al.,* 2017). Nyina-Wamwiza *et al.* (2010) noted that the lack of nutrient-rich feeds causes mortality and poor survival rate in African catfish which impedes the efforts to produce enough fingerlings to meet the high demand for fish. Optimum use of supplementary feed is important to obtain rapid tilapia growth (El-Sayed, 2006).

Dietary lipids contain fatty acids, phospholipids, sterols, and fat-soluble vitamins that are required for the smooth functioning of physiological processes as well as the maintaining of the biological structure and function of rabbit fish cell membranes to some extent (Ghanawei *et al.,* 2011). For maximum growth, Nile tilapia require more n-6 fatty acids than n-3 fatty acids (Aziza *et al.,* 2013), and they require about 1% of n-6 fatty acids in their diets (Bazaoglu & Bilguven, 2012). Squid extracted lipid and β-glucan positively affect the growth and the feed utilization when catfish are fed with high levels of polyunsaturated fatty

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acids (PUFAs) containing lipid (Lim *et al.,* 2001) and β-glucan in their diets and also provides sufficient energy for growth performance.

The length-weight relation is an essential component in fish biology. It is used to estimate the weight corresponding to a particular length and to identify potential significant differences between different unit stocks of the same species. It also provides data on Nile tilapia condition, growth patterns (Bagenal & Tesch, 1978), and health status (Ighwela *et al.,* 2011).

Catfish, notably Pabdah fish, *Ompok pabda*, are a major part of the fish fauna in Bangladeshi wetlands, and many of them are economically important due to their high nutritional value (Hossain *et al*., 2017; Tumpa *et al*., 2020). Despite its greater ecological and economic values, this species did not receive sufficient attention in aquaculture. Proper feeding and best management practices can enhance the production of Pabdah fish at farm levels. As the scanty number of fish in natural water bodies and the high mortality rate of the larvae are the major constraints for the successful aquaculture of this important fish species (Tumpa *et al.,* 2020), this study aimed to evaluate the effects of PUFAs and β-glucan containing diet on the growth performance, feed utilization, length-weight relationship, and condition factor of *Ompok pabda*.

MATERIALS AND METHODS

Experimental Diets

Two diets were maintained with 30% of protein levels. The experimental diet formulations consisted of without (control diet, CON) and with PUFAs containing total lipids and β-glucan (PBG diet) as shown in Table 1. In the PBG diet, squid lipids were used as the source of oil while soybean oil was used in the CON diet. Similarly, mushroom powder has been used as a source of beta-glucan in the PBG diet whereas wheat flour has been used in the CON diet, both of which are used as a source of carbohydrates. *Ganoderma lucidum* was used as a source of mushroom powder and maintained 1.2% β-glucan in the PBG diet whereas 12g β-glucan was present in 100g mushroom powder. All the dry ingredients (fish meal, rice bran, wheat bran, maize meal, vitamin B complex, and mushroom powder) were mixed using the Pearson square method for feed formulation. The diets were produced by blending the ingredients in a Hobart mixer with PUFAs, β-glucan, and distilled water, then pelleting the moist dough with a meat mincer with a 1-mm block. The 1-mm diameter pelleted two types of feeds were prepared from different ingredients viz., fish meal, rice bran, wheat bran, maize meal, vitamin B-complex, and mushroom powder (Table 1). Then the pellet feeds were kept in the tray and dried in the sun. After that, the feeds were allowed to store in the plastic bag in air-tight condition and kept in a dry place at room temperature for later use.

Experimental Setup and Collection of Fish

The feeding experiment was conducted on six cisterns, each sized (1.22 m × 2.44 m × 0.46 m) based on the number of dietary treatments arranged in triplicates. Each cistern was stocked with 60 healthy *Ompok pabda* collected from the wild with an average initial length and weight of 15.26 ± 0.36 cm and 16.45 ± 0.61 g, respectively. Water depth was maintained at 30 cm in each cistern throughout the study period. Individual fish were weighed using an electronic analytical balance at the beginning and end of the experiment (AND-GULF, Model-EK600, UAE).

Feeding and Sampling of Fish

The fish were fed twice a day with the formulated diets at 4% body weight to apparent satiation for 150 days. After feeding, uneaten diets were removed/siphoned from the cistern, left to dry, and re-weighted. Water was exchanged completely every 12 hours through the inlet and outlet system of the cisterns. The sampling was done once in two weeks to assess the growth and health status of the treated fish. We collected preliminary data on fish growth and feed consumption at the start of the feeding trial. After a 24-hour fast to clear the digestive tract and reduce handling stress, all fish were weighed at the end of the feeding trial. Fish were randomly captured from each cistern using a scoop net after the water level was lowered during sampling.

Physicochemical Parameters of Water

At weekly intervals, the temperature, dissolved oxygen (DO), pH, ammonia, total alkalinity, transparency, and salinity of the water in each cistern were measured. Temperature, dissolved oxygen (DO), pH, ammonia, total alkalinity, transparency, and salinity were measured by using a Celsius thermometer, a digital DO meter (multi 340 iset, DO-5509; China), a portable digital pH meter (MICRO-TEMP, pH 500, Romania), the API® ammonia test kit, a portable digital DO meter (MI 605, MARTINI) and refractometer (HANNA Instruments 96801, Romania), respectively.

Analysis of the Proximate Composition of Feed Ingredients

The protein content was measured by using Micro-Kjeldahl procedure. In the 2020 digester (Tecator),

Table 1. Percentage of formulated diet ingredients [a]

Ingredients	CON (%)	PBG (%)
Fish meal	26.5	26.5
Maize meal	20.5	20.5
Rice bran	21.5	21.5
Wheat bran	19.5	19.5
Wheat flour	10.0	0.0
Mushroom powder [b]	0.0	10.0
Soybean oil	1.0	0.0
Squid oil [c]	0.0	1.0
Vitamin B complex	1.00	1.00

[a] Used Pearson square method for feed formulations and maintained 30% protein level

[b] as a source of β-glucan

[c] as a source of PUFAs

weighed samples (approximately 0.5 g) were digested in a Kjeldahl flask with 10 ml concentrated sulfuric acid (H_2SO_4) in the presence of a catalyst (Kjeldahl tablet and selenium powder) for about 45 minutes at 420°C. The digested material was then distilled in the Kjeltech system 1026 distillation unit with a 33 percent sodium thiosulphate ($Na_2S_2O_3$) and a 40 percent sodium hydroxide (NaOH) solution. The distillate was collected in 25 ml of 4 percent boric acid (H_3BO_3), titrated with standard hydrochloric acid (HCl, 0.2 N), and the nitrogen value obtained was multiplied by 5.85 to get a percentage of protein. Moisture content was evaluated by drying a precisely weighed amount of sample (approximately 3 g) in pre-weighed porcelain for 24 hours at 105°C in an electric oven. Following weighing, samples (approximately 1 g) were placed in porcelain crucibles and heated in an oven at 110°C before ashing at 450°C overnight in a muffle furnace (Carbolite EML 11/6) and the average percentage of remaining material was taken as ash. Lipid content was measured using an all-ground-joined Soxhlet device to extract a weighed quantity of materials with analytical grade acetone. About 2.3 g sample was taken in pre-weighed thimbles and dipped in acetone. Extraction was allowed to proceed by heating in an Electromantle ME at 70°C until clean acetone (without oil) was observed in a siphon, which took around 3 hours, after which the apparatus' round bottom flask was separated and left for evaporation. Only the lipid remained in the flask when the acetone was evaporated, and this was later determined in percentages. A precisely weighed sample (about 0.5 g) was placed in a pre-weighed filter crucible, and crude fiber was assessed using a standard process before being estimated in percentage. By removing the amount of moisture, crude protein, ash, and crude fiber from 100, nitrogen free extracts were computed as a soluble carbohydrate using the following formula:

$$\text{NFE} = 100 - (\text{crude protein} + \text{fat} + \text{ash} + \text{moisture})$$

The results of analysis of the proximate composition of feed ingredients are shown in Table 2.

Analysis of the Fatty Acid Composition of Squid Meal

The samples were diluted in 2 ml n-hexane and then added to 0.4 ml methanolic 2 M NaOH solution for fatty acid proximate composition. The liquid was vortexed, then heated to 50°C for 2 minutes before adding 0.2 ml of methanolic 2 M HCl solution. With a 0.5 mm PEG-20 M liquid phase-coated 40 m 1.2 mm diameter G-300 column (Chemicals Evaluation and Research Institute, Saitama, Japan) and flame ionization detection, the n-hexane layer was collected, concentrated, and subjected to gas chromatographic examination. It was a Hitachi 163 gas chromatograph that was used (Hitachi Co. Ltd., Ibaraki, Japan). The column, detector, and injector were all heated to 170, 250, and 240°C, respectively. Fatty acid identity was determined by comparing peak retention times to authentic standards (St. Louis, MO, USA). DHA (22:6n-3) was determined to get a concentration of 39.3 percent, the highest among the fatty acids, while eicosapentaenoic acid (EPA) (20:5n-3) had a concentration of 11.2 percent, the second highest among the phospholipid acids. Palmitic acid (16:0) had the greatest percentage of saturated fats at 33.4 percent. Table 3 represents the total fatty acid composition.

Table 2. Proximate composition of feed ingredients and formulated feeds (% dry matter basis, mean ± SD)

Feed ingredients/ status	Ash (%)	Moisture (%)	Lipid (%)	Protein (%)	Carbohydrate and Fibre (%)
Fish meal	15.6 ± 2.7	13.4 ± 2.1	8.4 ± 1.6	62.4 ± 4.8	0.1 ± 0.02
Maize meal	3.7 ± 0.9	9.7 ± 2.3	5.7 ± 1.8	15.7 ± 3.2	54.8 ± 5.3
Rice bran	9.7 ± 2.4	13.4 ± 2.9	24.9 ± 3.6	17.7 ± 3.1	19.7 ± 3.4
Wheat bran	4.9 ± 1.1	10.6 ± 2.0	4.4 ± 0.9	14.5 ± 1.7	55.7 ± 3.7
Wheat flour	0.3 ± 0.02	11.9 ± 1.9	3.9 ± 0.7	10.9 ± 2.3	72.9 ± 4.3
Mushroom powder	1.2 ± 0.04	11.9 ± 1.8	2.4 ± 0.4	8.6 ± 1.9	75.8 ± 4.7
Control feed	7.8 ± 1.3	11.7 ± 2.6	11.0 ± 1.4	28.1 ± 3.6	33.6 ± 4.2
Treatment feed	7.9 ± 1.2	11.6 ± 2.8	10.9 ± 1.7	28.2 ± 3.9	33.3 ± 4.7

Table 3. Fatty acid composition of squid meal extraction

Fatty acids	Percentage (%)
C16:0, palmitic acid	33.4
C18:0, stearic acid	1.1
C18:1 (n-9), oleic acid	2.3
C18:2 (n-6), linoleic acid (LA)	0.9
C20:2 (n-6), eicosadienoic acid	0.4
C20:4 (n-6), arachidonic acid (ARA)	1.0
C20:5 (n-3), eicosapentaenoic acid (EPA)	11.2
C22:6 (n-3), docosahexanoic acid (DHA)	39.3
Others	10.4
Total	**100**

Estimation of Growth and Production Performance

The growth performance was determined by calculating the following formula.

$$\%\ \text{weight gain} = \frac{(\text{final weight} - \text{initial weight})}{(\text{initial weight})} \times 100$$

$$\%\ \text{length gain} = \frac{(\text{final length} - \text{initial length})}{(\text{initial length})} \times 100$$

$$\text{feed conversion ratio (FCR)} = \frac{\text{total feed intake (g)}}{\text{total wet weight gain (g)}}$$

$$\text{specific growth rate (SGR)} = \frac{(\text{In } W_f - \text{In } W_i)}{T} \times 100$$

where, W_f and W_i refer to the mean final weight and the mean initial weight, respectively, and T is the feeding trial period in days.

$$\text{FCE (\%)} = \frac{(\text{gain in wet weight in fish})}{(\text{feed fed})} \times 100$$

where: FCE = feed conversion efficiency

$$\text{HSI} = \frac{\text{liver weight (g)}}{\text{body weight (g)}} \times 100$$

where: HSI = hepatosomatic index

$$\text{KI} = \frac{(\text{liver weight (g)})}{(\text{body weight (g)})} \times 100$$

where: KI = kidney index

$$\text{VSI} = \frac{(\text{viscera weight (g)})}{(\text{body weight (g)})} \times 100$$

where: VCI = viscerosomatic index

$$\text{Survival rate (\%)} = \frac{(TF_f)}{TF_i} \times 100$$

where, TF_f is the total number of fish at finish (harvest) and TF_i is the total number of fish at initial.

Analysis of Length-Weight Relationship

Linear regression analysis was used to determine the length-weight (log-transformed) relationships, and scatter diagrams of length and weight were plotted. The length-weight relationship of the experimented fish is calculated using cube law given by Froese (2006).

The Length-weight relationship was estimated by using the equation:

$$W = aL^b$$

Where:
W = the weight of fish in grams
L = the total length of fish in centimeters
a = exponent describing the rate of change of weight with length (= the intercept of the regression line on the Y axis)
b = the slope of the regression line (also referred to as the allometric coefficient)

The log transformed data gave a regression equation.

$$\text{Log } w = \log a + b \log L$$

where;
a = constant
b = the regression co-efficient

Condition Factor

The value of the condition factor (K) was determined by the formula:

$$\text{Condition factor}: K = \left(\frac{W}{SL^3}\right) x\, 100$$

where 'W' is the mean weight (g) of fish and SL is the mean standard length (cm) of fish.

Statistical Analysis

Using a Microsoft Excel sheet on Windows 2010, the results were expressed as mean ± SD. The differences between control and treatment groups were analyzed using by paired-sample T-test using the statistical software package SPSS 22. The statistical significance was set at $P < 0.05$ and $P < 0.01$, as compared to the control group.

RESULTS AND DISCUSSION

Physicochemical Conditions of Water

During the entire experimental period, the physicochemical conditions of water did not vary significantly in the experimental cisterns (Table 4). The water quality of the present study was in good condition for appropriate health conditions and survival rates refer to Boyd & Tucker (1998); Pescod, (1985); Alabaster, (1982), etc.

Table 4. Physicochemical conditions of water during the experimental period

Parameters	Range (mean ± SD)
Temperature (°C)	26.5 ± 2.0
pH	7.4 ± 0.2
Dissolved oxygen (ppm)	6.7 ± 0.5
Ammonia	0.02 ± 0.0
Total alkalinity (mg/L)	120.30 ± 6.4
Transparency (cm)	25.20 ± 1.6
Salinity (ppt)	0.001 ± 0.0

Growth Performance and Feed Utilization

Body weight, length gain, feed conversion ratio (FCR), specific growth rate (SGR), and feed conversion efficiency (%) were significantly ($P < 0.01$) higher in fish fed with the PBG diet than in fish fed with the CON diet. The hepatosomatic index (HSI), kidney index (KI), and viscerosomatic index (VSI) (Table 5). The data of growth increment both in percentage weight gain and percentage length gain are presented in Figure 1. In this study, the survival rate was not affected by dietary treatments. Francis *et al.* (2019) found that PUFA-enriched *Artemia* fed Murray cod larvae shows a significantly higher SGR compared to the control group after 21 days of treatment.

Nonetheless, Bureau *et al.* (2008) found that dietary lipid and n-3 PUFA levels had minimal effect on rainbow trout, *Oncorhynchus mykiss*, growth, protein, and energy utilization. However, according to Zhou *et al.* (2011), both n-3 PUFA and a proper n-6/n-3 PUFA ratio are required for eel development and reproduction (*Monopterus albus*). Asdari *et al.* (2011) showed the effects of different dietary lipid sources on the growth performance of *Pangasianodon hypophthalmus* and the researchers found that the daily growth rate of fish was similar among fish fed with the soybean oil (SBO), crude palm oil (CPO), and linseed oil (LO) diets, but was significantly ($P < 0.05$) higher in the CPO compared to fish fed with the control (FO, fish oil) diet. Souza *et al.* (2020) observed that the effect of dietary β-glucan is not significant on different growth parameters namely weight gain, the daily weight gain, specific growth rate, apparent feed conversion, and condition factor but it is related to a good health condition, energy, and immune status of the fish body. As a result, the β-glucan is a

Table 5. Growth performance, feed utilization and somatic indices of *Ompok pabda* fed with the experimental diets for 150 days [a]

Parameters	CON	PBG
Mean weight of initial stock (g)	16.25 ± 0.53	16.45 ± 0.61
Mean weight of final stock (g)	54.56 ± 0.25	65.38 ± 0.43**
Mean length of initial stock (cm)	15.24 ± 0.41	15.26 ± 0.36
Mean length of final stock (cm)	22.11 ± 0.28	23.03 ± 0.39**
Feed conversion ratio (FCR)	2.34 ± 0.03	1.83 ± 0.02**
Specific growth rate (SGR)	0.668 ± 0.03	0.775 ± 0.02**
Feed conversion efficiency (%)	42.56 ± 0.43	54.64 ± 0.51**
Hepatosomatic index (HSI)	1.168 ± 0.012	1.218 ± 0.014*
Kidney index (KI)	0.328 ± 0.011	0.398 ± 0.013*
Viscerosomatic index (VSI)	1.543 ± 0.002	1.89 ± 0.003*
Survival rate (%)	100 ± 00	100 ± 00

Description: CON = control diet; PBG = polyunsaturated fatty acids (PUFAs) and β-glucan containing diet [a]Values of each parameter in the same row with asterisk are significantly different (***P* < 0.01and **P* < 0.05). Values are presented as mean ± SD of samples.

key bio-indicator of fish physiological status and a useful tool in assessing their energy. In the present study, the higher growth performance of fish fed with the PBG diet over the CON diet might be attributed to the supplementary effect of dietary PUFAs extracted from squids.

Length-Weight Relationship

Figures 2 and 3 show the length-weight relationship between pairs of plotted data, as well as the values of determination coefficients (R^2) and the corresponding equation. The value of the regression coefficient obtained from the length-weight relationship was 0.864 and 0.956 for fish fed with the CON and PBG diets. There was a strong relationship between length and weight. The values of correlation coefficients showed that there were highly significant relationships ($P < 0.01$) between the length and weight of the PBG diet group. Growth coefficients (b values) showed that the growth pattern of *O. pabda* was supposed to be isometric because the t-test showed that estimated b values had an insignificant deviation from the isometric growth pattern (b = 3). The length-weight relationships of fish fed

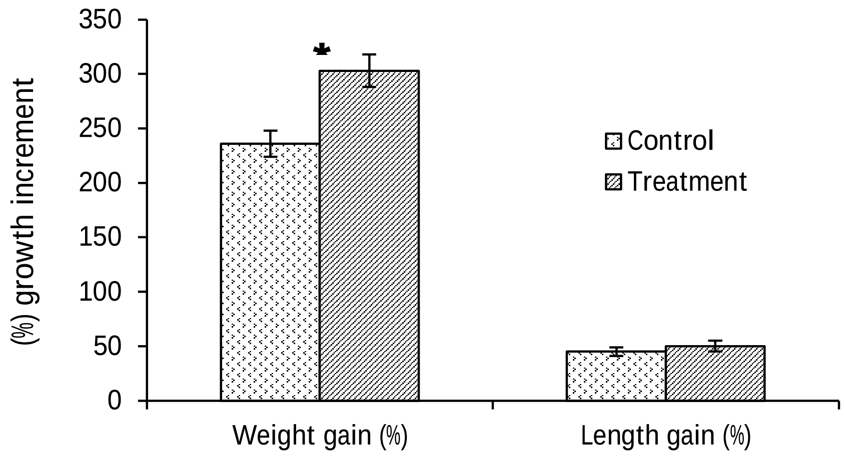

Figure 1. Growth performance observed of *O. pabda* in fish fed with the CON and PBG diets for 150 days. Vertical bars = mean ± SD. ($P < 0.01$).

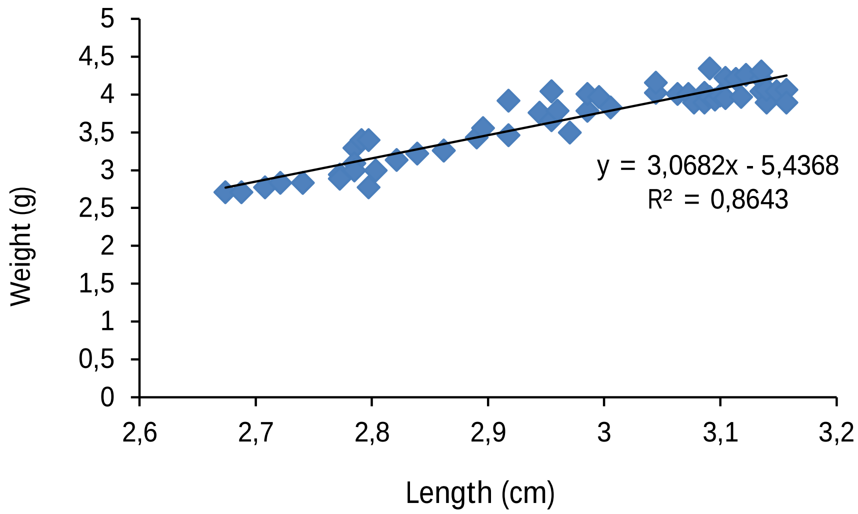

Figure 2. Length-weight relationship of *O. pabda* in fish fed with the CON diet for 150 days.

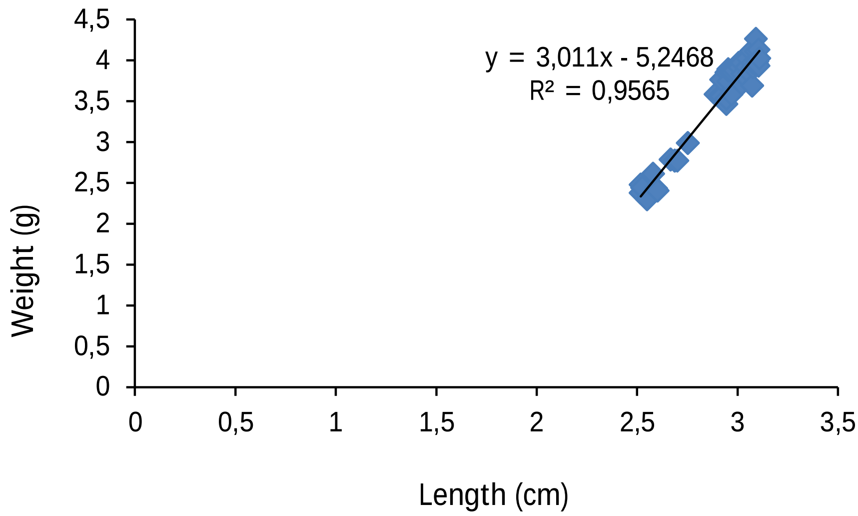

Figure 3. Length-weight relationship of *O. pabda* in fish fed with the PBG diet for 150 days.

with diets showed that the PBG diet group had better performance with supplemented diet than the CON group. The relevant results are shown in Table 5. The length-weight relationship is widely accepted as an important tool in aquaculture in terms of growth performance, feed utilization, population dynamics, and stock management (Abdoli *et al.,* 2008). Bwathondi & Abdulkarim (2017) found that the coefficient of determination (R^2) values in the CON and PBG groups with two different diets were 0.71 and 0.95, respectively of *Oreochromis niloticus,* while the PBG group significantly indicates good fitness. Datta *et al.* (2013) observed that the length-weight logarithmic relationship between length and weight with regression equation coefficient 'b' varied between 2.7675 to 4.3922 with different supplemental diets during the experimental period. All the fish in the dietary treatment groups did not show the same capacity to feed utilization. The length-weight relationship measures the variations in weight and length of individual fish groups and is an indication of fish growth and health status in aquaculture (Froese, 2006). Therefore, the length-weight relationships of *O. pabda* in the present study indicate that the growth performance of fish fed with the PBG diet with the supplementation of PUFAs and β-glucan was better than the CON group.

Condition Factor

The condition factor of fish fed the CON and PBG diets were 1.11 and 1.34, respectively during the study period. The condition factor of fish is an important parameter for understanding fish growth performance, biology, survival, reproduction, maturity, and health status of fish (Froese, 2006; Ridanovic *et al.,* 2015). It is frequently, used as a good indicator of water quality or the overall health of fish populations in a given area or ecosystem (Tsoumani *et al.,* 2006). Ridanovic *et al.* (2015) found that the condition factors of the tested fish were 0.922 and 0.967 with the control and treatment groups, respectively. Ighwela *et al.* (2011) observed that condition factors varied in *Oreochromis niloticus* when the fish was offered with four different levels of maltose in the diets which suggested that higher condition factor indicated good health condition during the experimental period and the results also demonstrated an isometric growth of the fish stock. Likewise, in the present study, the condition factor of the fish fed with the PBG diet showed significantly higher than that of the CON diet.

CONCLUSIONS

In the present study, the better growth were achieved by *O. pabda* fed with the PUFAs and β-glucan containing ingredients in terms of squid meal extraction and mushroom powder in the diet (PBG diet) ($P<0.01$). It can be corroborated that the PBG diet was very effective in improving the growth of the treated fish without any negative impact on the length-weight relationship and condition factor. The finding of the present study warranted further research on the utilization of different feed additives for *O. pabda* based on nutrigenomic approaches in the diet of this important commercial fish species.

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