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Length-weight Relationship and Condition Factor of Juvenile Nile Tilapia *Oreochromis niloticus* (Linnaeus 1758) Fed Diets with *Pyropia* spheroplasts in Closed Recirculating System

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Abstract

This study was conducted to assess the nutritional effects of *Pyropia* spheroplasts (PS) on the physical growth proportions of length-weight (L-W) relationship and condition factor (K) of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) in a closed recirculating system. Four different iso-energetic diets were prepared with different levels of PS (Diets 1 to 3) including a diet without PS as a control (Diet 4). Fish were fed with 38% protein diets and the results of this study showed that there was no significant difference (P>0.05) between groups according to protein intake. Experimental PS diets exhibited isometric (b=3) exponential growth, while control showed an allometric growth pattern (b<3). The coefficient of determination (r^2) of regression analysis was similar with all the dietary groups. The condition factor (K) varied from 1.2-1.7, while the highest value was observed in fish fed with Diet 3 (50 $g \cdot kg^{-1}$ PS) and the lowest was obtained in the control. Both regression coefficient (b) and condition factor indicated a proportional uniform growth pattern obtained in all PS levels. No negative effect was noted in the growth performance (P>0.05). Results showed that the PS can be used as fish feed ingredient for the proportional isometric growth of Nile tilapia.

Introduction

Substitution of fishmeal by plant alternatives is a necessity which is being driven by both economic and sustainability concerns while within the next decade fishmeal production may not be able to meet the required quantities for aquaculture. Intensified fishmeal requirement relies heavily on wild-caught fish.

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Due to supply insecurity of fishmeal there is an urgent need for sustainable protein sources to sustain the aquaculture industry (Hardy and Tacon 2002). In order to look for alternative protein sources, continuous efforts had been made by fish nutritionists and researchers over the past decades (Borquez et al. 2010). Various plant protein sources are found to be major candidates as ingredients in fish diets. However, these plant protein sources have some species specific drawbacks (Drew et al. 2007) such as high cost requirement and availability at industrial scale. According to Francis et al.(2001) plant-based ingredients have negative impacts on fish health as they contain anti-nutritional factors, such as protease inhibitors, trypsin and phytate, which reduce appetite and hinder protein digestibility. In recent years, marine macroalgae have been reported to be a potential protein substitute for aquaculture (Mustafa and Nakagawa 1995). Studies conducted on the use of algae as feed ingredients for fish reported significant improvement in fish body weight and length gain, as well as lipid composition (Xu et al. 1993; Mustafa et al. 1995). Among them the red alga *Pyropia yezoensis* (Ueda) M.S. Hwang & H.G. Choi 2011 is one of the important candidates that contains a higher percentage of protein, minerals and vitamins (Khan et al. 2008; Kalla et al. 2008; Sugita et al. 2009). The spheroplast isolation technique using polysaccharide-degrading enzymes has shown a positive promise that purple laver can be used as a food additive without the cell wall (Araki et al. 1994) because the cell wall of the alga is composed of three kinds of polysaccharides, β-1,4-mannan and β-1,3-xylan and porphyran, which are not easily digestible by aquatic species (Kalla et al. 2008). There are a few studies conducted on the use of fresh algae and the effects of the PS have not been broadly studied as feed ingredient for Nile tilapia.

The external traits like length-weight relationship and condition factors are important tools to study the biology of fishes (Lizama et al. 2002). These are used to predict weight from the proportion of length in a certain time (Pauly 1993). However, fish might show different growth patterns due to water quality, nutrition, habitat, density, sex and time of life stages (Ali et al. 2000). Thus length-weight relationship and condition factor are also important parameters for assessing the health condition (Nehemia et al. 2012; Mortuza and Al-Misned 2013). Many studies have been conducted to assess the length-weight relationship and most of them are related to stock assessment and aquatic environment (Baras et al. 2000; Baras et al. 2001), but, the prospective knowledge on the nutritional aspects is scanty. This study was conducted to assess the efficiency of PS on the external traits through length-weight relationship and condition factor, including body weight and growth in juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus 1758).

Materials and Methods

Experimental PS was prepared by using discoloured lavers *P. yezoensis* (Ueda). Three different kinds of cell wall degrading enzymes were used to breakdown the cell wall. Mixed enzymes (60 g β-1-4-mannanase, 200 units of agarase, 100 units of β-1-3-xylanase) were added in 30 L of water in a bucket and continuously stirred (1,100 rpm$\cdot$min^{-1}) at 20 ^{o}C. Four bundles of dried lavers (4 bundles×10 packs in each bundle×10 sheets in each pack×3 g of each sheet=1,200g) were immersed in the enzyme solution for 20 h.

Spheroplasts were collected by centrifuging at 2,000 rpm for 10 min, and PS was collected as a precipitate and immediately freeze-dried, ground into powder and used as a dietary feed ingredient in the experimental diets. The experimental diets were made of four different iso-nitrogenous formulations (Diets 1 to 4) with varying inclusion levels of PS at 10, 30, 50 $g{\cdot}kg^{-1}$ based on the nutritional requirements of Nile tilapia and Diet 4 was without PS as a control (Table 1). Diets were prepared using a 1.5 mm dye and dried for 48 h at 40 ^{o}C, and subsequently stored in an airtight polythene bag. Nile tilapia juveniles were obtained from Nikko River in Aichi prefecture of Japan.

Fish were acclimatised for 2 weeks and size graded to obtain fish of similar sizes. Twelve fish (mean initial body weight of 1.7 g) were randomly distributed into each of 12 (25×20×25 cm^3) rectangular plastic tanks at three replicates per treatment with a single water recirculating system. Water temperature was maintained at 27 ^{o}C with the use of a digital thermos controller system and photoperiod for 12L:12D using fluorescent light. The feeding schedule followed the protocol adapted from the National Research Council of US (1993). Fish were handfed three times daily for 5 weeks, thereafter they were anaesthetised with 450 ppm 2-phenozyethanol, weighed to the nearest 0.01 g using SP-401 balance. Klonk image measurement software was used to analyse the images for length measurements individually at weekly intervals. During the procedure the fish were kept for approximately 5 min in a 5 L plastic tank with well-aerated water while the respective chamber was being cleaned. Feeding ration was adjusted based on the weekly fish sampling. Water samples were monitored every week for total phosphorus (TP), ammonia- nitrogen (NH_3-N), nitrite- nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) using the HACH portable data logging colorimeter DR-850. Dissolved oxygen (DO) and pH were measured by using Lutron DO-5509 meter and HACH HQ40d model respectively.

Table 1. Composition of feed ingredients and proximate analysis of *Pyropia* spheroplasts and diets ($g{\cdot}kg^{-1}$) for juvenile Nile tilapia.

Ingredients	PS	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal		290	270	250	300
Soybean meal		350	350	350	350
Rice bran		100	100	100	100
Rice powder		50	50	50	50
Fish oil		80	80	80	80
Trace mineral premix		30	30	30	30
Vitamin premix		30	30	30	30
Carboxy methyl cellulose		50	50	50	50
Chromic oxide		10	10	10	10
Pyropia spheroplasts		10	30	50	0
Protein content ($g\ kg^{-1}$)	311	388	384	380	380
Lipid ($g\ kg^{-1}$)	82	123	121	118	125
Carbohydrate ($g\ kg^{-1}$)	466	275	281	284	283
Ash ($g\ kg^{-1}$)	61	119	117	114	121
Moisture ($g\ kg^{-1}$)	80	95	97	104	91

All chemical analyses followed AOAC procedures and were run in triplicates. Samples were analysed for moisture content (105 °C for 24 h), ash by combustion in a muffle furnace (550 °C for 6 h), crude protein (N×6.25) using a micro Kjeldahl method, lipid content by chloroform-methanol mixture method and carbohydrate was computed by 100- (Crude protein+Crude lipid+Crude ash+Moisture).

The L-W relationship (LWR) was computed using the equation $W = aL^b$ according to Pauly (1983).

Where, W = Weight of fish in gram (g), L = Total length of fish in centimetres (cm), a = Rate of change of weight with length (intercept), b = Weight at unit length

The equation was log transformed to estimate the parameters for ‘a’ and ‘b’. The log transformed data represented the regression equation.

$$Log\ W = \text{Log a + b Log L}$$

Where, a = Constant, b = Regression co-efficient

The growth is concluded as isometric when values of b are equivalent of three, otherwise allometric growth is concluded, which may be positive if >3 or negative <3 (Nehemia et al. 2012).

The degree of well-being of the fish was expressed by condition factor which was calculated by using the following equation.

$$K = 100\frac{W}{L^b}$$

Where, K = Condition factor, L = Total length of fish in centimetres (cm), b = Regression co-efficient obtained from length-weight equation.

The exponent ‘b’ value, that is equal $W = aL^b$ to 3, was not used to calculate the ‘K’ value. It was claimed that this exponent is not the real representation of the length-weight relationship for the great majority of fish species, therefore the ‘b’ value used here was obtained from the length-weight relationship equation (Lima-Junior et al. 2002). Correlation coefficient (r) was obtained from the coefficient of determination (r^2) and was checked for significance using the critical value of ‘r’.

Results

Water quality parameters showed that, the average DO values in the recirculating system was 6.7±0.16 $mg \cdot L^{-1}$. Dissolved oxygen values decreased over time, but stabilised at 6 $mg \cdot L^{-1}$. The average pH value was 7.2±0.08. The NH_3-N ranged from 0.04- 0.26 $mg \cdot L^{-1}$.

In the first week of the experiment, the NO_2-N was 0.27 mg·L^{-1}, and the value decreased with the progress of the study period. At the end of the experiment, NO_2-N was found to be 0.081 mg·L^{-1}, indicating that the bio filter system successfully denitrified the nitrogenous waste compounds to neutral form (Fig. 1a). With the progress of the experimental period, the total phosphorus (TP) level in the water recirculating system increased gradually. This result might be due to the increasing quantity of feed during the study period (Fig. 1b).

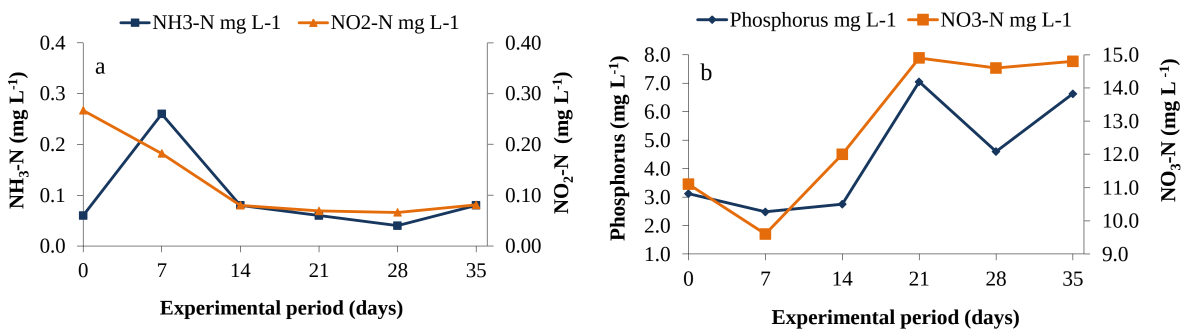

Fig. 1. Water quality parameters during the experimental period in recirculating system; (a) Ammonia nitrogen and nitrite nitrogen, (b) Nitrate nitrogen and phosphorus.

The chemical composition of the four formulated pellet diets and PS is shown in Table 1. The protein and lipid content in the experimental diets were not significantly different (P>0.05). Crude protein content in the experimental diets varied from 380 g·kg^{-1} to 388 g·kg^{-1} and crude lipid from 118 g·kg^{-1} to 125 g·kg^{-1}. The crude protein content in PS was 311 g·kg^{-1} but a comparatively higher proportion of carbohydrate was observed. Weekly protein intake did not show any significant differences among the groups (P>0.05). As the weight of the fish increased with time, the mean protein (g·week^{-1}) intake was increased as the study period progressed (Fig.2A). Higher average protein intake was observed in Diet 1 (10.16±0.1 g) and the lowest was observed in Diet 2 (9.43±0.42 g). Although mean lipid intake increased with the time of the study period, weekly lipid intake (g·week^{-1}) of the experimental groups was significantly different from the third week of the experiment (P<0.05). Higher lipid intake was observed in Diet 3 (3.33±0.08 g) (Fig.2B). Crude lipid content in the diets showed small variation among the treatments.

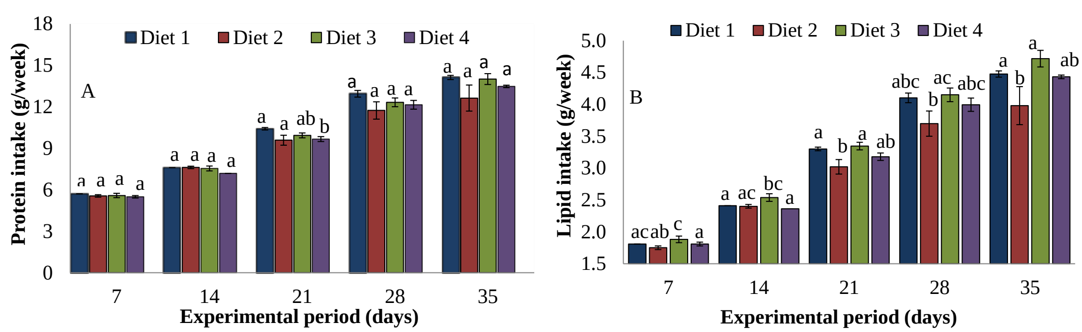

Fig. 2. Weekly variations in the protein (A) and lipid (B) intake of Nile tilapia. Data represent mean±SE. Bars having different letters on the same sampling times differ significantly.

A gradual increase of daily weight gain was shown in all the treatments (Fig.3). Weekly mean weight gain was not significantly different among the treatments (P>0.05). Satisfactory weight gain was found with the diets containing PS. The initial and final body parameters are presented in Table 2. The lowest mortality was observed at the higher level of PS diet. The mean initial weights of the fish during stocking were not significantly different among treatments. The highest individual final weight was observed in the PS diet groups (10.0±0.31 g) but this did not differ significantly from those fish given the control diet. Mean individual initial and final total lengths (cm) were not significantly different among the treatments. Initial total length ranged from 4.55±0.01- 4.69±0.07 cm and the final body height ranged from 2.52±0.04- 2.58±0.03 cm (P>0.05). The highest value was observed in Diet 3 and the lowest was in Diet 1 (Table 2). The proximate analysis of body muscle composition of the Nile tilapia is shown in Table 3. Highest crude protein content was observed in the fish fed diet with 30 $g^{.}kg^{-1}$ PS (Diet 3) and significantly different from 10 $g^{.}kg^{-1}$ PS diet (P<0.05). An increase in carcass ash percentage was observed with an increased PS supplementation level in this feeding trial (P<0.05). There was no difference among the crude lipid and carbohydrate content in the experimental fish.

Table 2. Mean value of body parameters of Nile tilapia cultured with different levels of *Pyropia* spheroplasts.

Treatment	Mortality rate (%)	Initial total length (cm)	Final total length (cm)	Initial body height (cm)	Final body height (cm)	Initial body weight (g)	Final body weight (g)
Diet 1	2.8±2.78	4.64±0.07	8.27±.08	1.37±0.02	2.52±0.04	1.8±0.01	10.0±0.31
Diet 2	8.3±4.81	4.72±0.03	8.08±0.14	1.41±0.03	2.54±0.05	1.8±0.05	9.8±0.27
Diet 3	0.0±0.00	4.68±0.05	7.94±0.16	1.39±0.00	2.58±0.03	1.8±0.05	9.7±0.25
Diet 4	2.8±2.78	4.55±0.01	8.26±0.07	1.36±0.00	2.57±0.01	1.7±0.02	9.4±0.20

Values are mean±SE of all fishes (n=12) in the triplicate tanks. Total length is the length of a fish measured from the tip of the snout to the tip of the longer lobe of the caudal fin. Body height is the greatest straight-line height or depth of the body, from the dorsal to the ventral surface.

Table 3. Proximate analysis of the experimental Nile tilapia at the end of the experiment ($g^{.}kg^{-1}$) on fresh matter basis).

	CP	CL	CA	Carbohydrate	Moisture
Diet 1	188.0±0.6^{b}	35.3±0.7	37.0±2.6^{b}	10.7±2.9	728.3±1.5
Diet 2	196.0±2.0^{a}	36.0±1.0	40.7±2.8^{ab}	7.3±3.4	720.0±0.0
Diet 3	196.3±3.2^{a}	35.7±1.2	45.0±2.1^{a}	5.7±2.2	717.3±6.5
Diet 4	193.0±3.0^{ba}	36.3±2.3	43.7±1.8^{ab}	4.3±0.7	722.7±3.8

n=3, mean values±SE, CA=Crude protein, CL=Crude lipid, CA=Crude ash.

The Nile tilapia showed an exponential growth in all the experimental diets. Exponential regression curve showed r^2 values of 0.99, and no significant differences were observed between the treatments (P>0.05) (Fig. 4). The regression curve indicated that PS with the cell wall digested diets did not show any negative results in the mean weight gain of Nile tilapia.

In this study, all the PS diets followed the cube law with maintaining the b value equal to 3 which exhibited an isometric pattern of growth. However, the control diet showed the allometric pattern of growth (b< 3), but the value was close to 3 (Table 4). The condition factor (K) varied from 1.2-1.7. The maximum 'K' value was found in the PS diet compared to the control diet. The correlation coefficient was significantly higher than the critical value (P<0.05). This indicated that the length-weight ratio was approximately similar and did not differ with different PS diets.

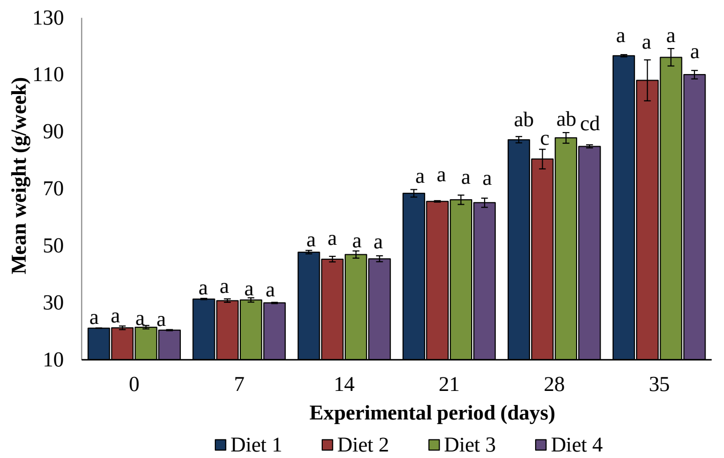

Fig. 3. Weekly mean weight gain of Nile tilapia in the close recirculating system during the experiment.

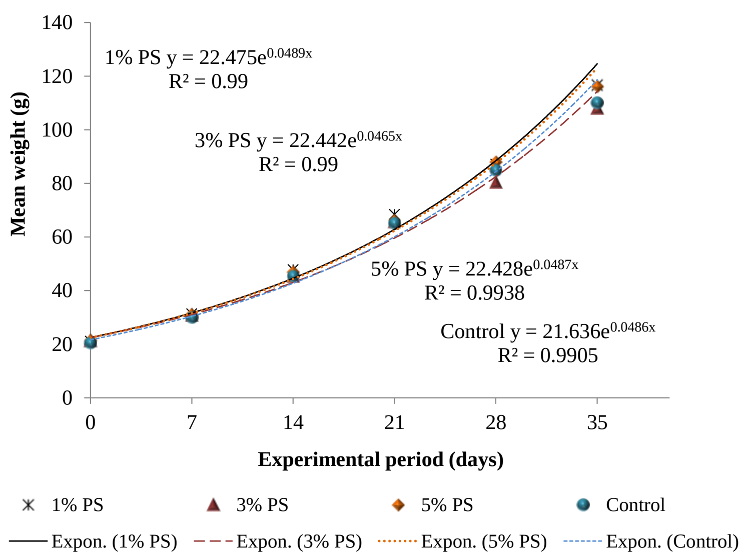

Fig. 4. Exponential growth curve of Nile tilapia fed different *Pyropia* spheroplasts diets in closed recirculating system.

Table 4. Equation parameters of length-weight and condition factors of Nile tilapia fed diets with *Pyropia* spheroplasts.

	Regression co-efficient (b)	Co-efficient of determination (r^2)	Correlation coefficient (r)	Condition factor (K)
Diet 1	3.0	0.93	0.96	1.3
Diet 2	3.0	0.93	0.96	1.7
Diet 3	3.0	0.93	0.96	1.7
Diet 4	2.8	0.93	0.97	1.2

Discussion

In this experiment, temperature was maintained at 27±1 ^oC. Though tilapia requires a low oxygen demand, in this experiment the average DO concentration was 6.7 ppm. Tilapia can survive a wide range of pH, and in the entire duration of the experiment, pH was 7.2, which ensured a neutral environment. Unionised NH_3 is highly toxic if the level is higher than 1.0 $mg \cdot L^{-1}$. The average NH_3-N was 0.1±0.03 $mg \cdot L^{-1}$.The level of nitrite was around 0.1 mg L^{-1}. The NH_3-N and NO_2-N decreased with the time of the experiment indicated the presence of *Nitrosomona*s and *Nitrobacter* in the biofilter system for converting NH_3-N to NO_2-N and NO_2-N to less toxic NO_3-N form (Timmons et al. 2002). The overall water quality parameters were consistent with the findings obtained by Cruz and Ridha (2011) using sinking and floating pellet diets for *O. niloticus* in the recirculating system.

According to Jauncey and Ross (1982) the optimum protein requirement is the level of high quality dietary protein needed for maximum growth. High protein diets will increase the waste output as well as increase the cost (Cho et al. 1994) of recirculating systems. Cruz and Ridha (2011) found a higher growth rate of Nile tilapia fed diets with crude protein content of 39- 42%. A similar result was obtained by Gur (1997), who reported that the daily weight gain at a crude protein level of 40% was significantly higher than at 30%. Stadtlander et al. (2013) found that the red alga *P. yezoensis* (Ueda) diet with 38.2% protein showed a higher growth rate for Nile tilapia compared to other treatments. In the present study, the level of protein was 38.0- 38.8%, thus the dietary feed protein present in the experimental diets were within the required level for Nile tilapia juveniles to support maximum growth. The results are in line with those of Loum et al. (2013) who reported that better growth was obtained with 38% crude protein for Nile tilapia fry reared in a recirculating system.

During the growth trial, tilapia grew gradually in all the treatments with the highest weight obtained on the 5^{th}week of the culture period. An exponential growth curve was shown that was similar to the studies done by Abdel-Tawwab et al. (2008) and Santos et al. (2013). The best result obtained from this study showed that the utilisation of PS as a dietary feed ingredient for tilapia juveniles was feasible in terms of overall growth performance and survival rate.

Studies evaluating the effect of dried seaweed (*Sargassum* spp.) as supplementary feed for Nile tilapia revealed that 5% of seaweed incorporated in the diet was sufficient to improve carcass quality (Yangthong et al. 2014). The use of 5% PS in the diets of red sea bream *Pagrus major* (Temminck and Schlegel 1843) did not show any significant difference in growth and feed utilisation (Kalla et al. 2008). In contrast, black sea bream *Acanthopagrus schlegeli* (Bleeker 1854) showed better growth performance using 3% of PS diet (Khan et al. 2008). It was suggested that 10% of *Porphyra dioica* J. Brodie & L.M. Irvine 1997 can be included in the diet of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Soler-Vila et al. 2009). Study on *Porphyra purpurea* (Roth) C. Agardh 1824 in artificial diets for thick-lipped grey mullet *Chelon labrosus* (Risso 1827) indicated that partial substitution of fish meal may prove to be cost effective (Davies et al. 1997). Moreover, it was observed that partial replacement of fish meal with red alga *P. yezoensis* (Ueda) improves growth and feed utilisation (Stadtlander et al. 2013). In the present study, carcass analysis of the PS trial did not show large variations, although some of the values were statistically different. Increasing the level of PS inclusion in the feed resulted in higher carcass protein content values. A lower percentage of carcass protein was found in the body muscle of Nile tilapia fed diet with 5% *Ulva* meal (Ergun et al. 2009). It was also reported that the red alga *P. dioica* inclusion level of 10% would not affect muscle protein deposition of rainbow trout (*O. mykiss*) (Soler-vila *et al*. 2009). Davies et al. (1997) observed an increase of crude protein in the carcass of thick-lipped grey mullet fed diet with red algae (*P. purpurea*).

In this experiment, PS diets showed isometric growth (b=3) and control diet showed allometric growth (b<3). It was reported that, if the value of ‘b’ is <3, it represents that the fish becomes less rotund with the increase of the length and if it is >3, it represents that fish becomes more rotund with the increase of its length (Kumari and Kumar 2015). The result of the experiment showed the healthy condition of the fish when they consumed the formulated diet at different levels of PS and fish grew equally in all directions compared to the control diet. It was advocated that the ‘b’ value usually lies between 2.5-4.0 (Ali et al. 2000). The regression coefficient of *O. niloticus* was found to be 2.9 for female, 3.2 for male and 3.1 for mixed-sex tilapia (Mortuza and Al-Misned 2013) which corresponded for positive allometric growth, whereas for *Oreochromis uroplepis* (Norman 1922) and *Tilapia zilli* (Gervais 1848) cultured in freshwater ponds showed negative allometric growth (Nehemia et al. 2012). In this experiment, the same age group of Nile tilapia juveniles were collected from the same stock and reared in the recirculating systems. The value of ‘b’ in the control diet was found to be lower, indicating that the fish was slimmer or contained less fat in their body.

The condition factor is an important factor to determine the relative degree of robustness and nourishment in fish (Mortuza and Al-Misned 2013). This factor might be influenced by sex, age, species type, maturity and environmental conditions (Anyanwu et al. 2007). Condition factor for all the experimental diets were >1 indicating that fish were above the average condition with good health during the entire period of the experiment. Higher value was observed in the PS diets as compared with the control.

This could be attributed to the accumulation of fat in the body (Laleye 2000). Lower value was observed in the control diet and that is a definite sign of allometric growth (Kumar et al. 2006). The condition factor for mixed-sex tilapia was found to be 1.09 (Mortuza and Al-Misned 2013) which was lower than the control diet of the present experiment. Condition factor of *O. niloticus* fed with different levels of maltose varied from 1.68- 1.86 (Ighwela et al. 2011) which was similar to the values obtained in the present experiment.

In terms of cost efficiency in commercially intensive tilapia farming, low-grade lavers could be a good source of feed ingredients for Nile tilapia. Low quality dry laver with no commercial value has become a serious environmental problem (Ishihara, et al. 2008) and effective uses of the waste laver have been sought. Therefore, unexploited seaweed and unused seaweed products could be used as a good source of additive in the fish diet (Yangthong et al. 2014).

Conclusion

Exponential growth curves were observed during the study. The length-weight relationship and condition factor values indicated the positive growth of *O. niloticus* in the closed re-circulating system. Fish fed diets with PS followed the cube law. This indicated that consuming PS diets, fish were in good condition and healthy and could provide good output in commercial aquaculture. It was concluded that there was a correlation between length and weight in fish fed diets with PS which could be used as a new cost effective feed ingredient in formulating commercial diets for Nile tilapia. Further research involving longer feeding trials and at higher inclusion levels of PS need to be carried out; the resulting biochemical composition of the fish and the apparent digestibility of the ingredients need also to be determined.

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