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GROWTH RESPONSE AND NUTRIENT UTILIZATION OF *Clarias gariepinus* JUVENILES FED TROPICAL KUDZU (*Pueraria phaseoloides*) LEAF MEAL INCLUSION DIETS

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ABSTRACT
A 10-week feeding experiment was conducted to evaluate the growth response and nutrient utilization of Clarias gariepinus juveniles fed Pueraria phaseoloides leaf meal (PLM) inclusion diets. Five isonitrogenous diets (D1-D5) were formulated to contain 40% crude protein with inclusion of PLM at 0%, 5%, 10%, 15% and 20% levels, respectively. D1 (0% PLM) served as the control diet. One hundred and fifty C. gariepinus juveniles of initial mean weight of 79.87 ± 5.85 g were randomly allocated to five dietary groups (D1-D5) and three replicates each and fed with the five PLM formulated diets, respectively, at 5% biomass daily. Fifteen concrete tanks of 1m^3 volume each were used for the experiment at ARAC. Each tank was stocked with 10 juveniles. Results showed no significant differences (p>0.05) in the mean weight gain, percentage weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, and nitrogen metabolism in all the dietary groups at the end of the feeding experiment. The present findings showed that PLM has good potentials for inclusion as a protein ingredient in C. gariepinus feed up to 20% level without reducing feed quality, nutrient utilization, and growth of fish.

Keywords: Aquaculture, fish, growth, nutrient, protein.

INTRODUCTION

Capture fisheries continue to decline globally (Gabriel *et al*., 2007) and as a result, researchers are grappling with an alternative source of food fish for the growing world population. Aquaculture seems to be a readily available alternative to the provision of food fish eaten in the world. Fish is widely accepted because it cuts across social, cultural, and religious backgrounds (Oresegun and Alegbeleye, 2001). Nutritionally, fish is one of the cheapest sources of proteins, essential fatty acids, vitamins, and minerals for millions of people in Africa (Bene and Heck, 2005). However, the lack of locally produced, affordable, and high-quality fish feed is one of the major challenges of aquaculture production in Nigeria and other parts of Africa (Gabriel *et al*., 2007).

Tropical kudzu (*Pueraria phaseoloides*) is a fast-growing plant species that belongs to the family *Fabaceae* and is used as a cover crop in the tropics. It is included in the Global Compendium of Weeds (Randall, 2012) and listed as one of the most aggressive weeds in the tropics (USDA-ARS, 2012). The growing season is all-year-round in the tropics and it does not have a dormant period. It is a twiner and climbs over other plants. It can become an invasive species (Soria *et al*., 2002) due to its fast growth, wide seed distribution, and ability to fully cover other plants. *P. phaseoloides* is ubiquitous and the leaves can easily be prepared as a leaf meal.

African catfish (*Clarias gariepinus*) belongs to the family *Clariidae* and is one of the most popular fish for culture in Nigeria (Adeogun *et al*., 2007). The reason for preference of the *Clariids* in tropical aquaculture includes hardiness to adverse environmental conditions, the fast growth rate in captivity, easy procurement of seed, adaptation to artificial feed, and high consumer preference in the market (Akinrotimi, 2008).

The present study seeks to investigate the suitability of inclusion of locally available and cheap *Pueraria phaseoloides* leaf meal as a protein ingredient in *Clarias gariepinus* diet.

MATERIALS AND METHODS

Collection and preparation of *Pueraria phaseoloides* leaf meal

The *P. phaseoloides* leaf sample was sourced and collected from the premises of African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. The sample was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt with Herbarium Number UPH/V/1460. The leaves were shade-dried at room temperature for three weeks and ground into very fine particles. The ground sample was stored for later usage.

Formulation and preparation of experimental diets

The feed ingredients used in this study were sourced locally from Modern Agro Enterprises, Rumuokoro, Port Harcourt, Rivers State, Nigeria. These include: wheat bran, soya bean meal, fish meal, bone meal, commercial fish premix, lysine, methionine, and vitamin C. Iodized salt, palm oil, and garri (binder) were purchased from Rumuokoro market. All the feed ingredients measured out summed up to 100% as shown in Table 1. Five isonitrogenous diets of 40% crude protein were formulated with varying inclusion levels of PLM. The control diet (D1) contained 0% PLM, D2 contained 5% PLM, D3 contained 10% PLM, D4 contained 15% PLM, and D5 contained 20% PLM. The feeds were formulated using Pearson Square method. The dough of each practical feed was pelletized separately using ARAC pelletizer through a 4mm die to produce pellets and sun-dried separately for 72 hours and more. The dry pellets were stored in air-tight plastic buckets and labeled accordingly.

Project location

The feeding trial was carried out at the African Regional Aquaculture Centre (ARAC), a Department of the Nigerian Institute for Oceanography and Marine Research (NIOMR), Aluu, Rivers State, Nigeria.

Proximate analyses

Proximate analyses of the dry leaf sample and the formulated diets were carried out at the Plant Anatomy and Physiology Research Laboratory, University of Port Harcourt, Rivers State, Nigeria. Moisture content, ash content, crude protein, crude fat, crude fibre, and total carbohydrates were determined. All analyses followed the Association of Official Analytical Chemists (AOAC) method (2006).

Water quality analyses

Water in all the experimental tanks was sourced from a borehole at ARAC Family Testing Unit. The water quality parameters determined include pH, temperature, dissolved oxygen, ammonia, nitrite, and total hardness. The temperature of the water was taken with a mercury-in-glass thermometer, while pH, dissolved oxygen, ammonia, nitrite, and total hardness were determined using LaMotte Fresh Water Aquaculture Test Kit (Code 3633-05, USA).

Source of experimental fish/acclimatization

One hundred and fifty (150) juveniles of *C. gariepinus* fish of mean weight 79.87 ± 5.85 g were obtained from the ARAC Catfish Hatchery. The fish were acclimatized for two weeks and fed twice daily with ARAC catfish feed at 5% biomass.

Experimental design, rearing units, and stocking of fish

The design of the experiment was a Completely Randomized Design with five treatment levels and three replicates each. A total of 15 concrete tanks of $1m^3$ volume each at the ARAC Family Testing Unit were used for the experiment. Each tank was stocked with 10 juveniles. A total of 150 juveniles were stocked.

Feeding of experimental fish

The juveniles were handfed twice daily at 09:00 hours and 16:00 hours. The daily ration of 5% biomass was divided into two; half fed to fish each time. The weight of feed fed was adjusted every two weeks to accommodate weight gain by fish. The fish were cultured for 10 weeks.

Data on weight changes were taken using a sensitive weighing scale (Camry Shadow Tools, China) while length increase was measured using Pentair Fish Measuring Board (Pentair Aquatic Eco-Systems, USA). The growth response and nutrient utilization by the experimental fish were calculated according to the following formulae after Rashid *et al.*, (2010):

Mean Weight Gain (MWG)

$$MWG = Mean\ Final\ Weight - Mean\ Initial\ Weight$$

Percentage Weight Gain (PWG)

$$PWG = \frac{Mean\ weight\ gain}{Mean\ initial\ weight} \times 100$$

Specific Growth Rate (SGR)

$$SGR = \frac{log_e W_2 - log_e W_1}{T} \times 100$$

Where:

W_2 = Mean final weight
W_1 = Mean initial weight
log_e = Natural log base e
T = Culture period (days)

Feed Conversion Ratio (FCR)

$$FCR = \frac{Feed\ fed\ (g)}{Weight\ gained\ (g)}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{Weight\ gain\ (g)}{Protein\ Intake\ (g)}$$

Where: $Protein\ Intake = \frac{\%\ crude\ protein\ in\ feed}{100} \times Feed\ Intake$

Nitrogen metabolism (NM)

$$NM = \frac{(0.54)(b-a)h}{2}$$

Where:

a = initial weight of fish
b = final weight of fish
h = experimental period in days
0.54 = experimental constant

DATA ANALYSIS

All values were reported as means ± standard deviations. The values of the various parameters were analyzed statistically by one-way analysis of variance (ANOVA) using SPSS Statistics version 21 software programme. LSD was used as Post Hoc Multiple Comparisons to identify which treatments were significantly different ($p < 0.05$) compared to the control.

RESULTS

Proximate composition of the leaf sample

The results of the proximate composition of dry *P. phaseoloides* leaf sample are presented in Table 2. Moisture content was 5.23 ± 0.23%; ash was 5.35 ± 0.75%; crude protein was 18.31 ± 2.62%; crude fat was 7.00 ± 0.00%; crude fibre was 43.96 ± 4.09%; and total carbohydrate was 20.15 ± 0.57%.

Proximate composition of the formulated diets

Table 3 describes the results of proximate composition of the five formulated diets. Moisture values ranged from 7.75 ± 1.61% to 10.34 ± 1.55%; ash ranged from 9.31 ± 0.36% to 11.35 ± 0.05%; crude protein ranged from 39.21 ± 0.29% to 41.44 ± 1.21%; crude fat ranged from 7.96 ± 0.65% to 9.45 ± 0.35%; crude fibre ranged from 5.12 ± 0.65% to 11.03 ± 1.19%; and carbohydrate ranged from 19.45 ± 2.19% to 26.96 ± 0.90%.

Physicochemical analysis of water in the experimental tanks

The water quality parameters in the experimental tanks indicated that pH values ranged from 6.61 ± 0.08 to 6.68 ± 0.02. Temperature ranged from 27.27 ± 0.01 ºC to 27.29 ± 0.01 ºC. Dissolved oxygen ranged from 6.59 ± 0.02 mg/l to 6.62 ± 0.01 mg/l. Ammonia ranged from 0.10 ± 0.01 mg/l to 0.12 ± 0.02 mg/l. Nitrite ranged from 0.29 ± 0.01 mg/l to 0.30 ± 0.02 mg/l. Total hardness ranged from 43.45 ± 0.01 mg/l to 43.48 ± 0.02 mg/l (Table 4).

Growth response and feed utilization of the experimental fish

Table 5 describes the mean values of growth response and feed utilization of *C. gariepinus* juveniles fed the *P. phaseoloides* leaf meal inclusion diets for 10 weeks. Mean weight gain (MWG) ranged from 269.06 ± 165.13g to 286.49 ± 168.58g; percentage weight gain (PWG) ranged from 341.43 ± 210.29% to 372.41 ± 257.77%; specific growth rate (SGR) ranged from 1.52 ± 0.25% to 1.58 ± 0.29%; feed conversion ratio (FCR) ranged from 1.26 ± 0.37 to 1.28 ± 0.40; protein efficiency ratio (PER) ranged from 2.10 ± 0.62 to 2.19 ± 0.76; while nitrogen metabolism (NM) ranged from 3840.11 ± 3587.85 to 4054.19 ± 3677.92.

Biweekly changes in mean weight gain of the experimental fish

Table 6 describes the biweekly changes in mean weight gain (MWG) of the experimental fish across all the dietary groups. Group 1 increased from 65.47 ± 6.55g in week 2 to 498.49 ± 20.17g in week 10; group 2 increased from 61.47 ± 4.74g to 485.03 ± 20.03g; group 3 increased from 59.00 ± 7.69g to 496.26 ± 19.31g; group 4 increased from 63.90 ± 4.03g to 505.50 ± 4.83g; while group 5 increased from 61.53 ± 4.65g to 511.87 ± 7.38g.

Biweekly changes in percentage weight gain of the experimental fish

The biweekly changes in percentage weight gain (PWG) of the experimental fish in all the dietary groups are shown in Table 7. Group 1 increased from 81.67 ± 7.46% in week 2 to 622.88 ± 46.49% in week 10; group 2 increased from 79.46 ± 6.47% to 627.49 ± 43.45%; group 3 increased from 75.18 ± 4.60% to 635.85 ± 40.96%; group 4 increased from 76.99 ± 4.10% to 611.03 ± 58.21%; and group 5 increased from 77.07 ± 5.98% to 741.87 ± 209.02%.

Biweekly changes in specific growth rate of the experimental fish

Table 8 describes the biweekly changes in specific growth rate (SGR) of the experimental fish across all the dietary groups. Group 1 decreased from 1.85 ± 0.12% in week 2 to 1.23 ± 0.04% in week 10; group 2 decreased from 1.81 ± 0.11% to 1.23 ± 0.04%; group 3 decreased from 1.74 ± 0.08% to 1.24 ± 0.04%; group 4 decreased from 1.77 ± 0.07% to 1.21 ± 0.05%; and group 5 decreased from 1.77 ± 0.11% to 1.25 ± 0.04%.

Biweekly changes in feed conversion ratio of the experimental fish

Table 9 shows the biweekly changes in feed conversion ratio (FCR) of the experimental fish in all the dietary groups. Group 1 increased from 0.86 ± 0.09 in week 2 to 1.88 ± 0.05 in week 10; group 2 increased from 0.89 ± 0.08 to 1.80 ± 0.03; group 3 increased from 0.93 ± 0.06 to 1.80 ± 0.03; group 4 increased from 0.91 ± 0.05 to 1.83 ± 0.06; while group 5 increased from 0.91 ± 0.07 to 1.77 ± 0.07.

Biweekly changes in protein efficiency ratio of the experimental fish

Table 10 shows the biweekly changes in protein efficiency ratio (PER) of the experimental fish in all the dietary groups. Group 1 decreased from 2.92 ± 0.27 in week 2 to 1.33 ± 0.03 in week 10; group 2 decreased from 2.83 ± 0.23 to 1.39 ± 0.03; group 3 decreased from 2.68 ± 0.17 to 1.39 ± 0.03; group 4 decreased from 2.75 ± 0.14 to 1.37 ± 0.05; while group 5 decreased from 2.75 ± 0.22 to 1.42 ± 0.06.

Biweekly changes in nitrogen metabolism of the experimental fish

Table 11 describes the biweekly changes in nitrogen metabolism (NM) of the experimental fish in all the dietary groups. Group 1 increased from 247.46 ± 24.77 in week 2 to 9421.53 ± 381.21 in week 10; group 2 increased from 232.34 ± 17.93 to

9167.00 ± 378.62; group 3 increased from 223.02 ± 29.08 to 9379.25 ± 364.99; group 4 increased from 241.54 ± 15.22 to 9553.95 ± 91.25; while group 5 increased from 232.60 ± 17.56 to 9674.28 ± 139.40.

DISCUSSION

Physicochemical analysis of water in the experimental tanks

Fishes completely depend on water for feeding, respiration, excretion, growth, reproduction, etc. Thus, a successful aquaculture business is dependent on good water quality management plus fish health. In the present study, the water quality parameters showed no significant differences (p>0.05) in all the experimental tanks. Therefore, whatever differences observed in the performance of the experimental fish were not a result of the culture water.

Proximate composition of the formulated diets

The moisture content, crude protein, and crude fat showed no significant differences (p>0.05) in all the experimental diets. Hence, the formulated diets were isonitrogenous and contained the same amount of crude protein, which is the most important nutrient in fish feed as it helps the fish to grow to its full potentials. However, ash content, crude fibre, and total carbohydrate varied significantly (p<0.05) across the dietary groups. In the present study, all the formulated diets were accepted by *C. gariepinus* juveniles, which indicated that the levels of inclusion of *P. phaseoloides* leaf meal did not affect the palatability of the diets.

Mean values of growth and feed utilization parameters

The mean values of all the growth and feed utilization parameters measured (mean weight gain, percentage weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, and nitrogen metabolism) showed no significant differences (p>0.05) across all the dietary groups at the end of the 10-week feeding experiment. This implied that the levels of inclusion of the *P. phaseoloides* leaf meal in the experimental diets did not compromise feed quality, nutrient utilization, and growth of fish.

Biweekly changes in the growth and feed utilization parameters

The mean weight gain (MWG), percentage weight gain (PWG), feed conversion ratio (FCR), and nitrogen metabolism (NM) increased from week 2 to week 10 in all the dietary groups; while specific growth rate (SGR) and protein efficiency ratio (PER) decreased from week 2 to week 10 across all the dietary groups.

Fish growth is measured in units of weight and length and is best presented as the specific growth rate (Ariweriokuma *et al*., 2016). According to Pohlenz *et al*. (2012), the growth rate decreased as fish size increased. In the present study, as fish size increased from week 2 to week 10, the specific growth rate decreased. Hence, the present study was in line with the findings of Pohlenz *et al*. (2012). Also, increased nitrogen metabolism across all the dietary groups indicated increased protein intake by the experimental fishes for metabolism into body mass; this agreed with the publication of Ariweriokuma *et al*. (2016).

PER is a measure of the efficient utilization of protein, while FCR is the quantity of feed needed to produce one unit of body weight. This implies that the lower the PER, and the higher the FCR, the poorer the feed utilization. In the present study, PER decreased while FCR increased as MWG increased from week 2 to week 10 across all the experimental groups. The results showed that feed/protein utilization into the fish body structure decreased as body weight gain increased. According to Dongmeza *et al*. (2009), feed utilization expressed as FCR and PER became less efficient with increasing body weight; because metabolic processes tend to diminish and energy expenditure per unit body weight declines with increasing body weight and age. Therefore, the present study agreed with the findings of Dongmeza *et al*., (2009).

CONCLUSION

In the present study, all the PLM inclusion diets were compared favorably to the control diet in all the nutrient utilization and growth parameters. Also, all the PLM formulated diets were accepted by *C. gariepinus* juveniles, which indicated that the levels of inclusion of the leaf meal did not affect the palatability of the feeds. Considering the all-year-round availability of *P. phaseoloides* leaf, its cheapness, high protein content, and the easy method of its preparation, the need to include PLM in the diets of *C. gariepinus* is highly imperative. Since the nutrient utilization and growth performance of fish fed D5 (containing 20% PLM) were as good as those fed the control diet (D1, containing 0% PLM), the results showed that PLM has good potentials for inclusion as protein ingredient in *C. gariepinus* feed up to 20% level without compromising feed quality, nutrient utilization, and growth of fish.

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Table 1: Percentage composition of experimental diets

Ingredients	Diet 1 (0% PLM)	Diet 2 (5% PLM)	Diet 3 (10% PLM)	Diet 4 (15% PLM)	Diet 5 (20% PLM)
PLM	0.00	5.00	10.00	15.00	20.00
Wheat Bran	19.76	15.02	10.27	5.54	0.79
Soybean Meal	33.80	33.67	33.54	33.41	33.28
Fish Meal	33.80	33.67	33.54	33.41	33.28
Palm Oil	5.00	5.00	5.00	5.00	5.00
Garri (Binder)	5.00	5.00	5.00	5.00	5.00
Bone Meal	1.50	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15
Vitamin C	0.10	0.10	0.10	0.10	0.10
Total (%)	100	100	100	100	100

Key: PLM – *Pueraria phaseoloides* leaf meal

Table 2: Proximate composition of *P. phaseoloides* leaf sample

Nutrients	% Composition
Moisture	5.23 ± 0.23
Ash	5.35 ± 0.75
Crude Protein	18.31 ± 2.62
Crude Fat	7.00 ± 0.00
Crude Fibre	43.96 ± 4.09
Carbohydrate	20.15 ± 0.57

Values are mean ± standard deviation of triplicate determinations.

Table 3: Proximate composition of the formulated diets

Sample Identity	% Moisture	% Ash	% Crude Protein	% Crude Fat	% Crude Fibre	% Carbohydrate
D1 (control)	7.75 ± 1.61[a]	11.35 ± 0.05[a]	39.37 ± 0.87[a]	9.45 ± 0.35[a]	5.12 ± 0.65[a]	26.96 ± 0.90[a]
D2	10.34 ± 1.55[a]	10.74 ± 0.06[a]	39.21 ± 0.29[a]	7.97 ± 0.15[a]	6.48 ± 3.02[a]	25.26 ± 1.54[a]
D3	9.37 ± 2.72[a]	9.91 ± 0.24[a]	41.07 ± 1.61[a]	7.96 ± 0.65[a]	7.79 ± 1.54[a]	23.90 ± 1.15[b]
D4	9.68 ± 0.00[a]	9.31 ± 0.36[b]	39.69 ± 1.31[a]	8.80 ± 0.70[a]	9.72 ± 0.98[b]	22.80 ± 1.13[b]
D5	9.04 ± 0.55[a]	10.76 ± 1.80[a]	41.44 ± 1.21[a]	8.28 ± 1.68[a]	11.03 ± 1.19[b]	19.45 ± 2.19[b]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (D1).

Table 4: Physicochemical analysis of water in the experimental tanks

Parameters	Experimental Tanks				
	Tank 1 (control)	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
pH	6.68 ± 0.02[a]	6.66 ± 0.01[a]	6.61 ± 0.08[a]	6.62 ± 0.03[a]	6.62 ± 0.02[a]
Temperature (ºC)	27.27 ± 0.01[b]	27.28 ± 0.01[b]	27.28 ± 0.02[b]	27.29 ± 0.01[b]	27.29 ± 0.01[b]
Dissolved Oxygen (mg/l)	6.62 ± 0.01[c]	6.59 ± 0.02[c]	6.60 ± 0.02[c]	6.59 ± 0.02[c]	6.60 ± 0.02[c]
Ammonia (mg/l)	0.10 ± 0.01[d]	0.11 ± 0.02[d]	0.12 ± 0.02[d]	0.12 ± 0.02[d]	0.12 ± 0.02[d]
Nitrite (mg/l)	0.29 ± 0.01[e]	0.29 ± 0.01[e]	0.29 ± 0.01[e]	0.30 ± 0.02[e]	0.29 ± 0.02[e]
Total Hardness (mg/l)	43.46 ± 0.02[f]	43.48 ± 0.02[f]	43.47 ± 0.02[f]	43.47 ± 0.03[f]	43.45 ± 0.01[f]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same row are not significantly different (p>0.05) compared to the control (Tank 1).

Table 5: Growth response and feed utilization of the experimental fish

Parameters	Experimental Groups				
	Group 1 (control)	**Group 2**	**Group 3**	**Group 4**	**Group 5**
MWG (g)	286.49±168.58[a]	269.06±165.13[a]	275.66±172.96[a]	282.57±173.92[a]	280.29±178.98[a]
PWG (%)	357.91±210.53[a]	348.18±213.68[a]	353.28±221.63[a]	341.43±210.29[a]	372.41±257.77[a]
SGR (%)	1.58±0.29[a]	1.54±0.26[a]	1.54±0.23[a]	1.52±0.25[a]	1.54±0.23[a]
FCR	1.27±0.45[a]	1.27±0.40[a]	1.26±0.38[a]	1.28±0.40[a]	1.26±0.37[a]
PER	2.19±0.76[a]	2.14±0.66[a]	2.13±0.60[a]	2.10±0.62[a]	2.13±0.58[a]
NM	4054.19±3677.92[a]	3840.11±3587.85[a]	3952.86±3701.54[a]	4035.78±3757.75[a]	4040.29±3820.90[a]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same row are not significantly different (p>0.05) compared to the control (Group 1).

Table 6: Biweekly changes in mean weight gain of the experimental fish

Experimental Groups	Mean Weight Gain (g)				
	Week 2	**Week 4**	**Week 6**	**Week 8**	**Week 10**
Group 1 (control)	65.47±6.55[a]	189.97±5.00[a]	289.23±9.08[a]	389.28±26.45[a]	498.49±20.17[a]
Group 2	61.47±4.74[a]	168.53±8.43[b]	265.20±13.95[b]	365.06±24.35[a]	485.03±20.03[a]
Group 3	59.00±7.69[a]	164.20±5.81[b]	275.33±6.96[a]	383.49±20.31[a]	496.26±19.31[a]
Group 4	63.90±4.03[a]	173.50±3.70[b]	279.80±5.42[a]	390.13±5.47[a]	505.50±4.83[a]
Group 5	61.53±4.65[a]	160.90±2.77[b]	275.63±9.67[a]	391.50±9.69[a]	511.87±7.38[a]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).

Table 7: Biweekly changes in percentage weight gain of the experimental fish

Experimental Groups	Percentage Weight Gain (%)				
	Week 2	Week 4	Week 6	Week 8	Week 10
Group 1 (control)	81.67±7.46[a]	237.60±20.65[a]	361.85±33.84[a]	485.55±27.05[a]	622.88±46.49[a]
Group 2	79.46±6.47[a]	218.11±17.49[a]	343.23±28.79[a]	472.59±46.03[a]	627.49±43.45[a]
Group 3	75.18±4.60[a]	210.92±22.13[a]	353.27±30.14[a]	491.19±32.75[a]	635.85±40.96[a]
Group 4	76.99±4.10[a]	209.63±18.89[a]	338.09±30.64[a]	471.40±42.06[a]	611.03±58.21[a]
Group 5	77.07±5.98[a]	207.03±13.32[a]	345.42±20.05[a]	490.66±26.82[a]	741.87±209.02[a]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).

Table 8: Biweekly changes in specific growth rate of the experimental fish

Experimental Groups	Specific Growth Rate (%)				
	Week 2	Week 4	Week 6	Week 8	Week 10
Group 1(control)	1.85 ± 0.12[a]	1.89 ± 0.10[a]	1.58 ± 0.08[a]	1.37 ± 0.04[a]	1.23 ± 0.04[a]
Group 2	1.81 ± 0.11[a]	1.79 ± 0.09[a]	1.54 ± 0.07[a]	1.35 ± 0.06[a]	1.23 ± 0.04[a]
Group 3	1.74 ± 0.08[a]	1.76 ± 0.11[a]	1.56 ± 0.07[a]	1.38 ± 0.04[a]	1.24 ± 0.04[a]
Group 4	1.77 ± 0.07[a]	1.75 ± 0.09[a]	1.53 ± 0.07[a]	1.35 ± 0.05[a]	1.21 ± 0.05[a]
Group 5	1.77 ± 0.11[a]	1.74 ± 0.07[a]	1.54 ± 0.05[a]	1.38 ± 0.03[a]	1.25 ± 0.04[a]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).

Table 9: Biweekly changes in feed conversion ratio of the experimental fish

Experimental Groups	Feed Conversion Ratio				
	Week 2	Week 4	Week 6	Week 8	Week 10
Group 1 (control)	0.86 ± 0.09[a]	0.84 ± 0.07[a]	1.20 ± 0.06[a]	1.56 ± 0.03[a]	1.88 ± 0.05[a]
Group 2	0.89 ± 0.08[a]	0.90 ± 0.08[a]	1.22 ± 0.06[a]	1.55 ± 0.08[a]	1.80 ± 0.03[a]
Group 3	0.93 ± 0.06[a]	0.92 ± 0.10[a]	1.16 ± 0.06[a]	1.49 ± 0.07[a]	1.80 ± 0.03[a]
Group 4	0.91 ± 0.05[a]	0.93 ± 0.07[a]	1.22 ± 0.06[a]	1.53 ± 0.06[a]	1.83 ± 0.06[a]
Group 5	0.91 ± 0.07[a]	0.94 ± 0.04[a]	1.19 ± 0.04[a]	1.47 ± 0.04[a]	1.77 ± 0.07[b]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).

Table 10: Biweekly changes in protein efficiency ratio of the experimental fish

Experimental Groups	Protein Efficiency Ratio				
	Week 2	Week 4	Week 6	Week 8	Week 10
Group 1 (control)	2.92 ± 0.27[a]	3.01 ± 0.24[a]	2.08 ± 0.11[a]	1.60 ± 0.04[a]	1.33 ± 0.03[a]
Group 2	2.83 ± 0.23[a]	2.79 ± 0.22[a]	2.05 ± 0.11[a]	1.62 ± 0.08[a]	1.39 ± 0.03[a]
Group 3	2.68 ± 0.17[a]	2.74 ± 0.29[a]	2.15 ± 0.11[a]	1.69 ± 0.08[a]	1.39 ± 0.03[a]
Group 4	2.75 ± 0.14[a]	2.70 ± 0.21[a]	2.06 ± 0.11[a]	1.64 ± 0.06[a]	1.37 ± 0.05[a]
Group 5	2.75 ± 0.22[a]	2.67 ± 0.12[a]	2.11 ± 0.07[a]	1.70 ± 0.05[a]	1.42 ± 0.06[b]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).

Table 11: Biweekly changes in nitrogen metabolism of the experimental fish

Experimental Groups	Nitrogen Metabolism				
	Week 2	Week 4	Week 6	Week 8	Week 10
Group 1(control)	247.46±24.77[a]	1436.15±37.82[a]	3279.91±103.02[a]	5885.92±399.90[a]	9421.53±381.21[a]
Group 2	232.34±17.93[a]	1274.11±63.73[b]	3007.37±158.22[b]	5519.71±368.23[a]	9167.00±378.62[a]
Group 3	223.02±29.08[a]	1241.35±43.95[b]	3122.28±78.94[a]	5798.42±307.11[a]	9379.25±364.99[a]
Group 4	241.54±15.22[a]	1311.66±28.01[b]	3172.93±61.52[a]	5898.82±82.74[a]	9553.95±91.25[a]
Group 5	232.60±17.56[a]	1249.42±70.05[b]	3125.68±109.68[a]	5919.48±146.57[a]	9674.28±139.40[a]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).