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Effect of protein deficient diets on the growth and carcass protein ash ratio of African catfish *Clarias gariepinus* (Burchell 1822)

***[1]NWANNA, LC; I. OMOJOLA[1]; E. NWANNA[2]; E. ABIODUN[1]**

**[1] Corresponding author., Department of Fisheries and Aquaculture Technology PMB 704 Akure, Nigeria. Phone: 234 8033582428. E-mail: drlel@yahoo.com. 2. Department of Biochemistry, FUT Akure.*

ABSTRACT: As the prices of fish feed ingredients continue to soar due to economic and environmental challenges, many fish farmers now produce practical fish diets deficient in protein, to minimize costs and maximize profits. This study evaluated the effects of such diets on the growth performance and carcass quality of African catfish, *Clarias gariepinus*. Five dietary treatments used contained 25%, 30%, 35%, 40% and 45% protein, respectively. Fish were fed for 70 days and the results showed high and improved growth performance in relation to the increasing dietary protein levels. Carcass protein, ash and minerals correlated positively with increasing dietary protein levels while the lipids had inverse relationship. Generally, fish fed diets of 25-35% protein had lower performance than those fed the control diets of 40 or 45% protein reported as the requirements for the fish. However, and in all cases, there were no differences in the performance of fish fed 40 or 45% protein diet, indicating no significant additional benefits from feeding the fish with 45% protein diet. The results also suggested the protein requirement of the fish to be above 35% and about 40%. The carcass protein-ash ratio was observed to be constant and ranged between 4.08 and 4.82. © JASEM

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Fish meal is the major ingredient in commercial fish feeds, especially those used for rearing carnivorous species (Nwanna, 2002), because it has balanced amino acid profile which makes it suitable as animal protein in fish nutrition. However, it is scarce and expensive, resulting in over 65% of the operating costs of aquaculture (Nwanna, 2002, Luo et al. 2004). Presently, about 11 million tones of fish constituting 12% of the total haul from seas and rivers are used each year to feed the farmed fish. Meanwhile, it takes 2 to 5 kg of wild fish just to produce 1 kg of a farmed fish such as salmon (Kendall 2003). Also in the recent time, climate change and unprecedented rain fall pattern and flooding have affected arable crops production negatively, resulting in scarcity of plant protein ingredients used in fish feed, thereby forcing the prices of available ones to double or triple especially in the tropical developing countries. Therefore in the face of declining capture fisheries and a booming aquaculture industry, many fish farmers have started cutting the costs of feed production by preparing practical diets deficient in proteins. As protein is the most important factor affecting growth performance of fish (Luo et al. 2004), it becomes necessary to study and document the effects of low protein diets on the growth of fishes. This study therefore investigated the effects of protein deficient diets on the growth, nutrient utilization and carcass quality of African catfish, *Clarias gariepinus*.

MATERIAL AND METHODS

Experimental animals and deign: Two hundred and fifty apparently healthy fingerlings of *C. gariepinus* (2.40-2.50 g) were acclimatized to the laboratory conditions for 15 days using five 700 L capacity flow-through fibre-reinforced plastic tanks with the provision of continuous aeration. During acclimatization, the fish were fed with a fish meal-based formulated diet (400 g protein kg). Fish were weighed individually and two hundred and twenty five uniform-sized healthy fish (2.40-2.50g) were equally distributed in five dietary treatment groups, T_1 (25% CP diet), T_2 (30% CP diet), T_3 (35% CP diet), T_4 (40% CP diet), T_5 (45% CP diet) with three replicates each and stocking density of 15 fish per tank in 150 L of rearing water, in a completely randomized design. The150 L capacity flow through glass tanks with a flow rate of 1.5 L min were used for rearing the fish. Tap water was used through out the experimental period. Treatments 4 and 5 served as the control as authors reported the protein requirement of the same size of *C. gariepinus* used in the present study to be either 40 or 45% (Fagbenro et al. 1992; Anyanwu 2000).

Feed preparation and feeding: In preparing the experimental diets, the required ingredients including fish meal, soyabean meal, groundnut cake, maize, fish/vegetable oil, methionine, vitamin mineral premix, and carboxymethyl cellulose were procured (Table 1). The major ingredients were blended into fine powder and sieved through a fine-meshed screen (0.5mm diameter). The required feed ingredients were mixed with carboxymethyl cellulose, vitamins and minerals and oil and thoroughly mixed together. Water was added and mixed with all the ingredients to make dough. Finally, the dough was pelletized using Hobart pelleting machine (Hobart Model 200, CA,USA) to get uniform size pellets (2 mm) and oven dried over night at 45^{o}C, the dried pellets were kept at -4^{o}C before use. Proximate compositions of

Crresponding Author* ***mail: drlel@yahoo.com*

the 5 diets were conducted according the AOAC (1995) standard methods (Table 2), while the gross energy of the diets was calculated as proposed by NRC (2011), based on physiological values of 4.11, 5.64 and 9.44 kcal/g (17.2, 23.6, and 39.5 KJ/g) for carbohydrates, proteins, and lipids, respectively (Table 2). All the treatment groups of fish were fed to apparent satiation thrice daily. Fish were weighed bi-weekly for calculation of the growth parameters. At the end of the experiment which lasted for 70 days, fish were counted and weighed. The growth parameters and feed utilization indices were calculated as follows: Weight gain = Final wt. – initial wt. Specific growth rate (SGR) = 100 (Ln W_2 − Ln W_1)/T; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period; Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g); Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g).

Carcass proximate composition and minerals analyses: At the end of the experiment fish were not fed for 24h. The catfish were weighed and five fish from each tank was collected. A total of 15 fish from each treatment were killed by anaesthetic overdose (ethylene-glycol-monophenyl-ether) (Liebert et al. 2006), oven dried at 48^{o}C (whole body) for 24h, blended into powder form and kept at -20^{o}C before use. Three replicates of the stored fish samples were analysed for proximate and minerals composition according to the standard methods of AOAC (1995).

Mineral analyses: Three replicates of the stored fish carcass were analysed for minerals according to the methods of AOAC (1995). About 2.0 g of the samples were ashed for 48 h at 480°C. After the ash had cooled to room temperature, 6 mL of 6 N HCl was added and the mixture was brought to boiling point. After cooling to room temperature, another 2.5 mL of 6 N HCl was added and the mixture was warmed to dissolve all the solutes. The solution was then cooled and diluted to 25 mL with distilled deionized water. Then the minerals (Ca, Mg, Zn, Mn) were measured in Atomic Absorption Spectrophotometer (AAS). P contents were determined using the vanadomolybophosphoric acid colorimetric method.

Statistical analyses: Comparison among the treatment means was carried out by one way analyses of variance (ANOVA) at (P=0.05) followed by Duncans multiple range test (Duncan 1955). The data were analyzed by SAS statistical package.

Table 1. Gross composition of experimental diets (g kg^{-1} DM)

Ingredients	Diet 1 25% CP	Diet 2 30% CP	Diet 3 35% CP	Diet 4 40% CP	Diet 5 45% CP
Fish meal	146	174.7	203.8	233	262.1
Soybean meal	160	190.5	221	254	285.1
Ground nut cake	175	210	246	280	316
Yellow maize	424	329.8	234.2	138	41.8
Fish/Veg oil	60.0	60.0	60.0	60.0	60.0
Vitamin-premix[1]	25.0	25.0	25.0	25.0	25.0
Carboxylmethyl cellulose	10.0	10.0	10.0	10.0	10.0

1.Kg^{-1} diet Kg^{-1} Vitamin and Minerals: Vitamin A –10,000,000 I.U.; D3- 2,000,000 I.U.; 23,000mg; K3 – 2,000 mg; B1 – 3,000 mg; B2- 6,000 mg; Nacin– 50,000 mg; Calcium Pathonate – 10,000 mg; B6 – 5,000 mg; B12- 25.0 mg; Folic acid 1,000 mg; Biotin- 50.0 mg; Choline chloride – 400,000 mg; Manganese – 120,000 mg; Iron- 100,000 mg; Copper– 8,500 mg; Iodine – 1,500 mg; Cobalt-300 mg; Selenium-120 mg; Antioxidant 120,000 mg.
E-23,000mg; K3-2,000mg; B1-3,000mg; B2-6,000mg; Nacin- 50,000mg;

Ca Pathonate-10,000mg; B6-5,000mg; B12-25mg; Folic acid-1,000mg; Biotin-50mg;

Choline chloride-400,000mg; Mn-120,000mg; Iron-100,000mg; Copper-8,500mg Iodine-1,500mg; Cobalt-300mg; Selenium-120mg; Antioxidant 120,000mg

Table 2. Proximate composition of experimental diets (% DM)

	Diet 1 25% CP	Diet 2 30% CP	Diet 3 35% CP	Diet 4 40% CP	Diet 5 45% CP
Dry matter	91.6	91.6	91.5	91.8	92.1
Crude ash	8.86	8.86	8.82	8.77	8.79
Crude fibre	6.00	6.05	5.99	6.55	6.10
Crude lipid	11.6	11.6	12.4	12.3	12.5
Crude protein	25.1	30.2	35.1	40.1	45.2
GE (KJ g^{-1})	18.5	18.9	19.0	19.3	19.6

Table 3. Growth and nutrient utilization of *C. gariepinus* fed the experimental diets

	Diet 1 25% CP	Diet 2 30% CP	Diet 3 35% CP	Diet 4 40% CP	Diet 5 45% CP
Initial wt. (g)	2.50	2.40	2.40	2.40	2.50
Final wt. (g)	18.8^{c}	24.3^{b}	28.4^{b}	50.7^{a}	57.4^{a}
Weight gain (g)	16.3^{c}±3.27	21.9^{b}±5.60	26.0^{b}±3.40	48.3^{a}±5.99	54.9^{a}±9.05
SGR	2.88^{bc}±0.28	3.33^{ab}±0.22	3.53^{a}±0.15	4.36^{a}±0.18	4.48^{a}±0.23
FCR	2.54^{a}±0.54	1.76^{a}±0.16	1.63^{a}±0.20	1.10^{b}±0.15	1.01^{b}±0.17
PER	15.6^{b}±2.85	17.1^{b}±4.10	19.4^{b}±4.67	24.7^{a}±4.04	26.7^{a}±2.08

Values of 3 replicates on the same row with same superscript are not different (P>0.05)

Table 4. Proximate composition of *C. gariepinus* fed the experimental diets.

	Diet 1 25% CP	Diet 2 30% CP	Diet 3 35% CP	Diet 4 40% CP	Diet 5 45% CP
Dry matter	90.2^{a} ±1.32	90.2^{a}±1.32	91.0^{a}±0.92	91.1^{a}±1.76	91.2^{a}±0.12
Protein	55.4^{a} ±0.37	55.9^{a}±0.92	59.5^{a}±0.64	60.4^{a}±0.61	64.9^{a}±0.18
Lipids	15.7^{a}±2.11	15.0^{a}±1.83	14.6^{a}±1.94	13.8^{a}±1.61	12.6^{a}±1.56
Ash	11.5^{b}±0.29	12.6^{b}±0.31	14.0^{a}±0.61	14.8^{a}±0.96	15.6^{a}±0.51
Protein: ash	4.82^{a}±0.39	4.44^{a}±0.51	4.25^{a}±0.67	4.08^{a}±0.26	4.16^{a}±0.21

Values of 3 replicates on the same row with same superscript are not different (P>0.05)

Table 5. Minerals composition of *C. gariepinus* fed the experimental diets (mg g^{-1} DM).

	Diet 1 25% CP	Diet 2 30% CP	Diet 3 35% CP	Diet 4 40% CP	Diet 5 45% CP
Ca	24.3^{b} ±4.25	28.2^{b}±7.93	31.5^{a}±1.02	33.5^{a}±1.23	36.6^{a}±0.12
P	11.9^{b}±0.16	12.0^{b}±0.21	19.8^{a}±0.05	21.9^{a}±0.36	23.9^{a}±0.05
Mg	2.41^{ab}±0.04	3.93^{ab}±0.14	4.44^{a}±0.07	4.97^{a}±0.09	5.02^{a}±0.51
Zn	0.13^{b}±0.01	0.14^{b}±0.03	0.15^{a}±0.06	0.17^{a}±0.03	0.30^{a}±0.15
Mn	0.08^{b}±0.06	0.09^{b}±0.10	0.12^{ab}±0.05	0.16^{a}±0.00	0.25^{a}±0.01

Values of 3 replicates on the same row with same superscript are not different (P>0.05)

RESULTS AND DISCUSSION

The proximate composition of the experimental diets (Table 2) showed closely related values according to dietary formulations. The protein contents of the diets increased accordingly resulting to marginal increases in the gross energy of the diets.

The growth and nutrient utilization of the fish (Table 3) indicated that the fish fed 25-35% dietary protein retarded the mean weight gain, feed conversion ratio and protein efficiency ratio of the fish significantly. The specific growth rate (SGR) increased with increasing dietary protein levels and there were no significant differences in the SGR of the fish fed diets 3, 4 and 5. SGR of the fish fed diet 3 was also marginally higher than that of the fish fed diet 2 but significantly higher than that of the fish fed diet 1. The feed conversion ratio (FCR) and protein efficiency ratio (PER) improved with increasing levels of dietary protein.

Proximate composition of the fish carcass (Table 4) indicated that the protein and ash contents of the fish increased with increasing dietary protein levels, but while there were no differences (P>0.05) in protein levels of fish in all the treatments, significant differences existed in the carcass ash. Fish fed diets 3, 4 and 5 contained the same (P>0.05) quantity of ash which are higher (P<0.05) than those of the fish fed diets 1 and 2. Lipid contents of the fish showed a declining trend with increasing levels of protein in the diets, but there were no significant differences in the composition. The carcass protein and ash ratio (protein: ash) was calculated to test the hypothesis that they are constant in mammals, birds and fishes, and the results obtained seem to support that.

Generally, the minerals contents of the fish (Table 5) increased with increasing dietary protein levels. Carcass Ca was statistically the same in the fish fed diets 3, 4 and 5, but their values were significantly higher than those in the fish fed diets 1 and 2. Carcass P and Zn followed the same trend as in Ca, while there were no differences (P>0.05) in the Mg and Mn contents of the fish.

Protein as the most essential nutrient for fish growth and maintenance of physiological functions is costly and takes between 50 and 70% of the total feed costs (Nwanna, 2002). Therefore many fish farmers in a bid to reduce feed costs usually produce diets deficient in proteins. This study investigated the effects of such diets on the growth and carcass quality of African catfish. Observation from the present study indicated clearly that in inadequate dietary protein retarded fish growth and nutrient utilization. Fagbenro et al., (1992) reported the protein requirement of *C. gariepinus*, as 40%, while Anyanwu (2000) reported 45% for the same species. Adebayo (2005) similarly reported the protein requirement of hybrid catfish as 45%. The results from this study confirm 40% protein as the requirement for *C. gariepinus* and also showed that increasing the dietary protein content of the fish to 45% did not have any statistical advantage. This is in line with the report of Oishi et al. (2010) which stated that excess protein does not support additional increase in growth performance, but rather results in economic losses and deterioration of water quality.

In the present study, carcass protein and ash contents of the fish were positively correlated with dietary protein levels, while the lipid content of the fish had inverse relationship with dietary protein levels. Similar results on carcass body protein of other species of fish have been reported (Liebert et al., 2006). Sardar et al. (2009) also reported increased nitrogen retention and decreased lipid retention in Indian major carp with both supplemental Met and lysine and additive effects using a combination of both amino acids. The observed inverse relationship between the carcass lipid and dietary protein levels has also been noted with other fish species (Oishi et al., 2010). However, this observation is in contrast with that of Shiau and Lan (1996) who reported positive correlation between carcass lipid of grouper *Epinephelus malabaricus* and dietary protein levels. They also opined that differences in carcass lipid content might be attributed to differences in the dietary carbohydrates as fish differ in their ability to utilize carbohydrates.

The mineral composition of the experimental fish explains the increasing levels of carcass ash with increasing levels of dietary protein. The calcium and phosphorus contents of the fishes made up about 80 - 90% of the total minerals thus making them the dominant inorganic components in the whole fish body. This observation strongly supports the work of Hertrampf and Piedad-Pascual (2000). Skeletal mineralization affects the level of ash and hence the mineral contents in animals (Clawson et al. 1991). The hypothesis that protein ash ratio in birds, mammals and fishes is constant was well demonstrated in the present study. This is in contrast with the documentation of Clawson et al. (1991) which stated that the protein:ash ratio in the whole body of representative mammals, birds and fishes is not constant but subject to the effects of nutrition, physiological state, sex, genetics and age of the animal. They further explained that, in some cases of amino acids or protein deficiency, the protein:ash ratio increased relative to the control because the dietary Ca or P intake or absorption limited skeletal mineralization. However, this focus of research needs further investigation to establish how skeletal development and protein:ash ratio in the body of animals is regulated.

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