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Growth Responses of Hybrid Catfish (*Hetero-clarias*) Juveniles Fed All Plant: Protein Diets Supplemented with L-lysine and L-methionine

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Abstract

A 70 day feeding trial was conducted to evaluate the utilization of all plant- protein diets supplemented with L-lysine and L-methionine by Hybrid catfish *(Hetero-clarias)* juveniles. At the start of the experiment, fifteen (15) glass aquaria tanks (70 cm × 45 cm × 40 cm)/each, filled with borehole water up to 70 L of its capacity were stocked with one hundred (150) Hetero-clarias juveniles (Av. Wt. 16.49 ± 0.02 g)/one at 30 fish per treatment in three replicates in a complete randomized design. Five diets (D_1-D_5) isonitrogenous (40% Crude Protein) and isocaloric (12.1kcal/kg) were formulated. Except the control (D_1), varying levels of Corn Gluten Meal (CGM) of 60% crude protein (CP) as main plant protein source were added to other plant protein materials such as Soya bean meal and Groundnut cake to formulate diets (D_2-D_5). Fish were fed at 5% body weight twice daily (8.00 and 10:00) for 10 weeks. Water quality parameters were monitored daily. Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) were determined. Proximate and mineral composition of the diets and fish were determined using AOAC methods. Fish fed D_1 (control) significantly ($P<0.05$) had the best growth performance in terms of final mean weight gain (44.54 ± 0.02)g; Specific Growth Rate (1.86 ± 0.06) and Protein Efficiency Ratio (1.11 ± 0.04). The mean value for Feed Conversion Ratio (FCR) was significantly ($P<0.05$) highest (1.4 ± 0.04) in fish fed. Least mean value (8.00 ± 0.03) for Net Protein Utilization (NPU) was also recorded in fish fed D5. Fish fed D2 with minimal plant materials exhibited feed utilization close to D1 (100%FM) as reflected in the growth performance. Fish growth was significantly impaired from (D3-D5) despite supplementation with amino acids, suggesting that inclusion of all-plant protein materials in the diets of Hetero-clarias at this stage of growth should not be encouraged.

Keywords: All-plant; Protein materials; Hetero-clarias; Diets; L-lysine; Growth responses

Introduction

Nutritional need of fish at every stage of growth is very important in other to formulate feeds that are nutritionally balanced with optimum utilization by the species, particularly when they are cultured under intensive system [1]. A diet is not complete without the presence of all the classes of food such as carbohydrate, protein, energy, minerals, fat and oil, and vitamin. Several research works had attempted at increasing the use of non- conventional feed materials to substitute for the high cost of fish meal, which is a major source of protein in fish diets [2-5]. Some of the factors that determine the protein level and source in fish diets are the size of fish and species [5]. It is important to accurately balance the protein need of a fish especially at early stage of development for optimum feed utilization. Over the years, the quest for alternative protein source to fish meal has been a growing concern in aquaculture industries due to high cost of fish meal. Corn gluten meal (CGM) is considered a good and palatable source of protein in fish diets, but it is deficient in lysine and methionine [6]. It is often supplemented with other plant protein materials such as soybean meal and groundnut cake to complement its amino acid profile [7]. Generally, fish feeds prepared with all-plant protein materials have poor amino acid profile hence the need to supplement with methionine and lysine, been the most limiting amino acids in plant feed materials [8]. This is necessary to achieve optimal growth and health of the fish. Hybrid catfish (*Hetero-clarias)* feeding habit is adaptable, though it exhibits cannibalistic tendency as growth progresses, its protein requirement must not be compromised at early growing stage. It grows well on formulated feeds as well as living or dead materials in the water. Because of its wide mouth at adulthood, it is able to swallow relatively large prey in water. Hybrid catfish requires balance diets of high protein content at juvenile stage, hence the need to use protein materials that are rich, readily available and cheap. The objective of the study was to evaluate the effects of all-plant protein materials supplemented with L-lysine and L-methionine in the diets of *Hetero-clarias* juveniles on growth and body proximate composition at the end of the feeding trial.

Materials and Methods

Experimental procedure

The study was conducted at the laboratory of the Department of Fisheries and Aquaculture Management, Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Nigeria. Prior to the start of the experiment, 200 juveniles of *Hetero-clarias* (hybrid) of average initial weight of 16.00g were purchased from Success Fish Breeding and Poultry Farm Nig, Ltd. Akure, Ondo State. Fish were kept in a flow- through concrete tank of 4 × 2 x 1m size for two weeks and fed with Coppens (imported feed) for acclimatization to experimental conditions. 150 juveniles fish out of the 200 were randomly selected and bulk weighed using an electronic top loading balance and randomly stocked into fifteen (15) glass tanks (70 × 45 × 40 cm)/one earlier filled with bore hole water up to 70 litres capacity. In line with the set objectives, five treatments representing the number of diets including the control were replicated thrice in a completely randomized design

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with ten fish/replicate. Fish were made to empty their gut content after a day starvation (24 h) before administering the test diets to make them receptive to the new diets and were fed for 70 days.

Experimental diets

All ingredients used in feed preparation were purchased from Metrovet Feed Mill, Ado-Ekiti, Nigeria. Ingredients were analysed for proximate composition prior to feed formulation [9]. The gross composition of the experimental diets is shown in Table 1. In preparing the diets, only diet (D_1), the Control had fish meal as protein source, while diets (D_2-$D_{5)}$) contained various proportions of corn gluten meal, soya bean meal and ground nut cake as protein sources at a ratio of 2:1:0.5 respectively. Dry ingredients were ground to a powdery form (250µm) in a Wiley mill to aid assimilation by fish. The diets were thoroughly mixed with cod liver oil and then adding cold water (500ml) until stiff dough resulted. The dough was pelleted using Hobart A -200 pelleting machine with a 2.0mm die. Diets were immediately sun dried and later broken mechanically into small sizes and packed in a labelled air tight small containers. Each diet was formulated to last for a month to maintain good quality. Except the Control (D_1), diets were isonitrogenous (40%CP) and isocaloric (12.1kcal/kg) as recommended by Faturoti et al. (1986) for clariid catfishes juveniles.

Feeding procedure

Fish were fed to satiation twice daily in two equal installments between 08:00-10:00 and 16:00-18:00 for 70 days. Initial and final weights of the fish in each tank were recorded using an electronic balance (Metler PM 480, Delta range). Fish were bulk-weighed every 14 days to calculate their response to feed. Good water quality was maintained throughout the feeding period by arranging the experimental tanks in a flow –through system while air stones were used to aerate the tanks.

Water quality analysis

Water quality parameters such as dissolved oxygen (DO), pH, temperature and nitrate were monitored and measured daily throughout the experimental period by the method of Boyd and Tucker (1992). (DO and temperature: YSI, model 57, USA; pH: Electronic pH meter (metler Toledo 320 model). Nitrate was measure using a DREL/5 spectrophotometer.

Growth and nutrient utilization parameters

Growth responses were calculated as described by Olvera- Novoa et al. (1990) as follows:

Weight gain (g)=final body weight – initial body weight

SGR (%)=100($\log_e$ final body weight- $\log_e$ initial body weight/time (days)

FCR=(dry weight of feed fed (g)/ fish weight gain (g)

PER=(fish weight gain (g)/ protein fed (g)

NPU (%)=100 × (Fish protein gain/ protein consumed)

Proximate analysis of diets and fish

The five diets and fish samples (whole body) from each treatment were prepared in triplicates after the experiment and analyzed using standard methods of the Association of Official Analytical Chemists for protein, fat, ash and moisture [9]. Crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction and ash was determined by combusting samples in a muffle furnace at 600°C for 5 h. Moisture content was estimated by heating samples in an air oven at 105°C for 24 h and computing weight loss

Statistical analysis

Data obtained (Mean weight gain, SGR, FCR, PER, NPU) from the feeding trial were subjected to analysis of Variance (ANOVA) using SPSS version 16. Differences in significant means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

Results

Proximate composition of experimental diets

The gross composition of the experimental diets is presented in Table 2. Crude protein ranged between 40.19% and 40.50% while crude fibre ranged between 1.06% and 1.67%. Also, lipid, ash and nitrogen free extract (NFE) varied from 6.36% to 7.32%, 6.71% to 7.14% and 31.88% to 34.18%, respectively. There was no significant difference ($p>0.05$) in the values of the parameters analysed among the treatments. This shows that the diets were formulated in line with the set objectives. Therefore any changes in the performance of the fish were assumed to be the effect of the all-plant protein diets supplemented with amino acids.

Water analysis

The result of the physico-chemical parameters of the experimental rearing water is presented in Table 3. Water temperature fluctuated within the range of 25.36-25.81°C thoughout the feeding trial. While

Ingredients	Diets				
	D_1	D_2	D_3	D_4	D_5
Fish meal	95	-	-	-	-
Corn gluten meal	-	40	42	44	46
Soya bean meal	-	20	21	22	23
G/Nut cake	-	10	10.5	11	11.5
Wheat meal	-	21.5	18	14.5	11
L-Methionine	-	1.5	1.5	1.5	1.5
L- Lysine	-	2	2	2	2
Vit/Premix	2	2	2	2	2
Cod liver oil	1.5	1.5	1.5	1.5	1.5
Cellulose	1.5	1.5	1.5	1.5	1.5
Total (%)	100	100	100	100	100
CP Cal. (%)	55	40	40	40	40
Kcal/kg	12.1	12.1	12.1	12.1	12.1

Table 1: Gross composition of experimental diets fed to hetero-clarias juveniles (%).

mean values obtained for dissolve oxgen (DO), pH and nitrate ranged between 5.20-5.29 mg/L, 6.81-6.91 and 0.20-0.22 mg/L respectively. There was no significant difference ($p>0.05$) in the values of the parameters analysed among the treatments.

Growth performance and nutrient utilization parameters

The growth parameter of the fish fed with the experimental diets is presented in Table 4. There were significant differences ($p<0.05$) in growth performance of the fish. Mean weight gain showed similar pattern to that of SGR, with the best values in fish fed D_1 (44.58 g, 1.86) respectively. Feed utilization, expressed as the feed conversion ratio (FCR), was significantly ($p>0.05$) different in all the treatments with the least mean value (1.07) in fish fed diets D_1 (the control). The dietary protein utilization, expressed as the protein efficiency ratio (PER) and net protein utilization (NPU) were significantly highest ($p<0.05$) in fish fed D_1 (1.11, 10.10%) respectively. The results further indicated a progressively diminished growth rate from D_2 to D_5.

Proximate composition of Experimental Fish after the feeding trial

Table 5 shows the result of the proximate analysis of the experimental fish after the feeding trial. There was a significant difference ($p>0.05$) in Protein content of fish carcass fed D_1 to D_5 with the highest value (63.50%) in the control treatment (D_1). Fat content in fish carcass was inversely related to protein content. That is, fish fed D_5 had the highest lipid content (18.87%) and least (12.05%) in D_1. There was a similar result in carcass ash with the highest values in D_5 (15.52%) as plant protein materials levels in diets increases. There was a gradual increase in NFE content in fish carcass. Fish fed D_1 (1.18%) had the lowest NFE content and highest in fish fed D_5 (3.25%)

Discussion

Fishmeal is the preferred protein source in the diets of fish, especially the young ones. It serves as a good source of animal protein with an appreciable amount of energy, lipid, minerals, valuable phospholipids,

Parameters			**Diets**		
	D_1	D_2	D_3	D_4	D_5
Moisture content (%)	11.17	11.76	11.27	10.22	11.98
Ash (%)	7.14	7.3	6.87	6.71	6.98
Lipid (%)	6.49	7.32	6.36	7.14	6.78
Protein (%)	40.33	40.5	40.25	40.19	40.33
Crude fibre (%)	1.06	1.24	1.23	1.56	1.67
Nitrogen free extract (%)	33.81	31.88	34.02	34.18	32.26

Table 2: Chemical composition of experimental diets fed to hetero-clarias (hybrid) juveniles.

Parameters	Treatments				
	D_1	D_2	D_3	D_4	D_5
Temp.^{0}C	25.74 ± 0.45	25.79 ± 0.45	25.81 ± 0.45	25.77 ± 0.45	25.36 ± 0.45
P^H	6.81 ± 0.21	6.91 ± 0.21	6.91 ± 0.21	6.87 ± 0.21	6.85 ± 0.21
DO_2 (mg/L)	5.22 ± 0.16	5.25.22 ± 0.16	5.265.22 ± 0.16	5.235.22 ± 0.16	5.295.22 ± 0.16
NO_3(mg/L)	0.2 ± 0.001	0.22 ± 0.001	0.21 ± 0.001	0.21 ± 0.001	0.22 ± 0.001

No significant difference ($P > 0.05$) in the values of the parameters analysed.

Table 3: Mean Values of water quality parameters in all treatments for hetero-clarias juveniles.

Parameters	Treatments				
	D_1	D_2	D_3	D_4	D_5
Initial mean weight(g)	16.45 ± 0.04^a	16.41 ± 0.02^a	16.46 ± 0.03^a	16.45 ± 0.04^a	16.44 ± 0.04^a
Final mean weight (g)	61.03 ± 0.06^a	47.07 ± 0.06^b	45.94 ± 0.06^c	45.01 ± 0.06^c	32.71 ± 0.04^d
Mean weight gain (g)	44.58 ± 0.02^a	30.66 ± 0.02^b	29.48 ± 0.02^b	28.56 ± 0.02^c	16.27 ± 0.02^d
SGR%	1.86 ± 0.06^a	1.48 ± 0.01^b	1.48 ± 0.01^b	1.46 ± 0.04^c	0.98 ± 0.01^d
FCR	1.07 ± 0.03^d	1.10 ± 0.01^c	1.11 ± 0.06^c	1.34 ± 0.04^b	1.40 ± 0.04^a
PER	1.11 ± 0.04^a	0.75 ± 0.03^b	0.72 ± 0.03^b	0.53 ± 0.03^c	0.52 ± 0.06^c
NPU%	10.10 ± 0.03^a	10.00 ± 0.02^a	9.14 ± 0.06^b	8.27 ± 0.01^c	8.00 ± 0.04^c

Mean values with the same superscripts along vertical row are not significantly different $P > 0.05$.

Table 4: Growth performance and nutrients utilization of hetero-clarias juveniles fed with experimental diets.

	Treatments				
Sample %	D_1	D2	D_3	D_4	D_5
%moisture	$10.18^c \pm 0.02$	$12.63^a \pm 0.01$	$11.57^b \pm 0.10$	$12.60^a \pm 0.05$	$12.76^a \pm 0.01$
% Ash	$13.09^e \pm 0.09$	$13.31^d \pm 0.11$	$13.78^c \pm 0.10$	$14.83^b \pm 0.04$	$15.52^a \pm 0.03$
% Lipid	$12.05^e \pm 0.02$	$13.87^d \pm 0.01$	$15.83^c \pm 0.03$	$16.50^b \pm 0.04$	$18.87^a \pm 0.10$
% Protein	$63.50^a \pm 0.11$	$58.50^b \pm 0.01$	$56.85^c \pm 0.01$	$53.15^d \pm 0.12$	$49.60^e \pm 0.02$
NFE	$1.18^d \pm 0.03$	$1.69^c \pm 0.04$	$1.97^c \pm 0.03$	$2.92^b \pm 0.02$	$3.25^a \pm 0.01$

Mean values with similar superscript along the horizontal row are not significantly different $P > 0.05$

Table 5: Proximate composition of experimental fish after the feeding trial.

fat-soluble vitamins, and steroid hormones. Another very important reason why fishmeal is sought after as an ingredient in fish diets is because of its acceptability and palatability. Also, fishmeal is an excellent source of calcium and phosphorus to fish. The main limiting factor is the high cost, which affects its usage level in fish feed formulation [6].

Plant protein sources such as Soya bean meal and ground nut cake contain some anti-nutritional factors such as trypsin inhibitors [10]. Though, these anti-nutritional materials can be removed by heat during processing, it does not make the plant nutrients to be one hundred percent readily available to fish. Plant protein sources generally have poor amino acids profile, hence the need to supplement it with most limiting amino acids in fish diets. In this study it was observed that despite the high crude protein level in corn gluten meal and other plant protein materials, the diets were poorly utilised by the Hetero-clarias Juveniles as reflected in the growth performance. This findings support the claim by Enyidi and Mgbenka [4], that young *Clarias gariepinus* growth rate was impaired with high inclusion levels of plant material as replacement for fish meal in their diets. In this study, fat in fish carcass increased with increase in plant materials in the diets. The implication of this is that, according to Adebayo and Aladejare [5], growth of fish would be suppressed as seen in this study, due to poor intake of feed.

Fish farm with an intensive management system of production requires diets of high crude protein content to feed the young fish. This consideration is important in view of the fact that fish feed account for between 60-70% of the operating cost [11]. Increased understanding of the nutritional requirement of a particular fish species would allowed the use of both fish meal and plant materials to formulate low cost diet that would supplement natural feeds in larviculture [12-16].

Conclusion

Feed formulation involves substituting one feedstuff for another and compound feed at the lowest cost. Without compromising growth and feed utilization, this study established that Corn Gluten Meal with other plant protein materials such as soya beans Meal and ground nut cake should be used at minimal level in the diets of *Hetero-clarias* juveniles. All-plant protein materials offered a better alternative protein sources in fish diets since they are readily available and cheap. Currently in fish farms, fish feed is expensive due to high cost of feed ingredients, especially the protein sources for the young ones. The only cheap and sustainable alternative is to promote the use of locally available plant materials to replace expensive fish meal in fish diets.

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