# EFFECT OF ALL PLANT-PROTEIN DIETS SUPPLEMENTED WITH L-METHIONINE AND L-LYSINE ON GROWTH PERFORMANCE OF *HETEROBRANCHUS BIDORSALIS* JUVENILES

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## Abstract

*A feeding trial was conducted to investigate the growth performance of Heterobranchus bidorsalis fingerlings fed all-plant-protein diets supplemented with two limiting amino acids. At the start of the experiment, ten glass aquaria tanks of size 70cm x 45cm x 40cm volume/one were stocked with 150 juveniles of Heterobranchus bidorsalis mean weight (6.8±0.1g) at 30 fish per treatment in three replicates for five treatments including the control, using completely randomized design. Except for the Control ($D_1$), fish were fed with all-plant-protein diets ($D_2$-$D_5$) containing corn gluten meal, soybean meal and Groundnut cake for 70 ays. Fish were fed twice per day at 10:00 and 18:00 hours respectively. It was observed that fish fed with $D_1$ significantly ($P<0.05$) had the best growth performance in terms of Net Protein utilization (8.33±0.06), Specific Growth Rate (1.17±0.06), Final Mean Weight (60.10±0.04) and Protein Efficiency Ratio (0.86±0.06). Feed Conversion Ratio was highest in fish fed $D_5$ (3.11±0.02) with the poorest growth performance. It could be deduced from the results that juveniles of Heterobranchus bidorsalis requires the minimal inclusion of plant –protein materials in its diets for optimum growth.*

**Keywords:** *Heterobranchus bidorsalis*, juveniles, plant-protein, growth performance

## Introduction

Nutrition is the intake of food in relation to body dietary needs. Poor nutrition impaired growth and increased susceptibility to infections (Whitney and Rolfes, 2005). Diets must contain all necessary nutritional requirements for fish to ensure the best growth performance. Balance diet for fish must include all classes of food such as carbohydrate, protein, lipids, vitamins, minerals, and water. Protein is the most expensive feed material and must be adequately supplied in fish diets for optimal growth (Zehia and Khan, 2011). Increasing protein level in fish diets leads to high cost of fish production, hence the need to use low-cost protein materials in fish diets (Adebayo and Aladejare, 2014). In addition, minimal use of fish meal in fish diets is necessary to reduce the cost of feeding which accounts for over 60% of operating expenses. Possible replacement of fish meal with plant-protein materials would reduce the cost of feed production without compromising optimal growth of fish (Sotolu, 2010).

Corn gluten meal is considered to be one of the most suitable alternative ingredients for replacing fish meal in fish diets. Studies have shown successful replacement of fish meal with corn gluten meal without any adverse effect on growth (Otubusin, *et al*, 2009).Corn gluten is plant-protein rich ingredients, containing about 65% crude protein, used as a source of protein and energy for fish species (RFA, 2008). Though, its amino acid profile is poor in lysine and methionine; hence the need to supplement the diets with these essential amino acids during feed formulation for fish. In order to ensure maximal growth, fish species need ten (10) indispensable amino acids in their diets (Ghomi *et al,* 2012). Mai *et al*, (2006) confirmed that lysine is often the most limiting amino acid ingredients used to prepare fish diets. Lysine is not only involved in protein synthesis but also along with methionine which serves as the precursor of carnitine which carries fatty acids across mitochondrial and facilitates their oxidation to produce energy and consequently increase growth performance (Deng *et al*, 2010).Methionine also takes part in protein synthesis and other important physiological functions in the body of aquatic animals (Zhou *et al*, 2006).

*Heterobranchus bidorsalis* is widely cultured in Nigeria due to its outstanding characteristics such as an ability to utilize various types of locally formulated feeds and to withstand unfavorable environmental conditions (Owodeinde and Ndimele, 2011).The aim of the study was to substitute fishmeal with corn gluten meal and other plant materials supplemented with L-Methionine and L-Lysine in the diets of *Heterobranchus bidorsalis* juveniles by establishing appropriate supplementation level with the best growth performance.

## Materials and Methods

### Experimental Setup and Procedure

The experiment was carried out at the Faculty of Agricultural Sciences, Ekiti State University, Ado- Ekiti. Prior to the start of the experiment, 150 juveniles of *Heterobranchus bidorsalis* (6.52$\pm$0.5)g/one were purchased from Success Fish Farm Nigeria Limited, Akure, Ondo State Nigeria. Fish were kept in a concrete pond filled with clean water for two weeks to acclimatize them and to reduce their stress. During this period, fish were fed with imported fish feed (2mm) once a day. The fish were weighed after two weeks of acclimatization with electric sensitive scale before they were stocked into ten plastic tanks (70cm x 45cm x 40cm). Five treatments including the control were replicated thrice in a complete randomized design at ten fish per replicate and fed for 70days with the formulated diets

### Experimental Diets

The whole ingredients used in feed preparation were purchased from a reputable feed mill in Ado- Ekiti, Ekiti State. Ingredients were analyzed for proximate composition prior to feed formulation according to (AOAC, 2012). The gross composition of the experimental diets is represented in Table 1. In the process of preparing the diets, dried ingredients were ground to a powdery form at the Afe Babalola Feed Mill, Ado Ekiti. The diets were thoroughly mixed with cod- liver oil and pelleted using Hobart, A 200T pelleting machine with a 2.0mm die. The pelleted diets were sun-dried and packed into labeled sack prior to use.

**Table 1**: Gross composition of experimental diets fed to *Heterobranchus bidorsalis* juveniles (%)

| 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 |

**Feeding Procedure**

Fish were fed once daily at 18:00 for 70 days while the weight of the experimental fish was measured bi-weekly to calculate their response to feed. Water was changed every three days to allow freshness of water and maintain good water quality throughout the time of the experiment.

**Water Quality Parameters**

The physicochemical parameters of the water were taken during the feeding trial. The PH values of the water were measured directly by an electronic PH meter (Metler Toledo 300 model) by dipping the electrode into each tank. Dissolve oxygen level was measured using standardized YSI dissolved oxygen meter (YSI model 57), the water temperature was measured using mercury in glass thermometer. Measurements were carried out gently by immersing the thermometer into the water at the vertical position and left for about 2-5 minutes and was quickly moved near the surface of the water and read.

**Growth Parameters**

In order to calculate the growth parameters and predict a daily food ration, growth performance and feed conversion were measured in terms of individual fish weight, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU). Growth response parameters were calculated as follows:

Specific growth rate (SGR) % =

$$100 \times \frac{(\text{In}Wf - \text{In}Wi)}{T}$$

Where In is the natural log

Wf is the final weight of the fish at time (t)

Wi is the initial weight of the fish at time 0

T is the trial period or culture period

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Net weight gain (g)}}{\text{Amount of protein fed (g)}}$$

$$\text{Net protein utilization (NPU)} = 100 \times \frac{(\text{protein gain})}{(\text{Protein consumed})}$$

Mean weight gain (MWG) = Final mean weight – Initial mean weight

**Chemical Analysis**

Samples of experimental diet, initial and final carcasses in all treatments were analyzed for proximate composition, moisture, ash, crude fiber, crude protein, crude fat and carbohydrate. Gross energy (kcal/kg) of the diet was determined using ballistic oxygen bomb calori-meter (Gallenkamp) as described by AOAC (2005).

### Statistical Analysis

Data obtained from the mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), net protein utilization (NPU) and proximate composition of the fish were subjected to analysis of variance (ANOVA) followed by Duncan multiple range test for significant values using SPSS version 9.1, values were considered significant at 5% level of significance.

### Results

### Water Quality Parameters during the feeding trial

The mean value of the physico-chemical parameters of water in the five treatments is presented in Table 3. Mean values for temperature were in the range of 25..38mg/l in $D_5$ to 25.81mg/l in $D_2$. Mean PH range from 6.83mg/l in $D_5$ to 6.93mg/l in $D_2$, while mean values of Dissolved oxygen range between 5.18mg/l in $D_2$ to 5.31mg/l in $D_5$. These measured values were not significantly different (P>0.05) in all the treatments.

**Table 3:** Mean values of water quality parameters during the feeding trial

| Diets | Parameters | | |
|---|---|---|---|
| | Temperature ($^0$C) | pH | DO (mg/l) |
| $D_1$ | 25.72±0.45 | 6.87±0.21 | 5.20±0.16 |
| $D_2$ | 25.81±0.45 | 6.93±0.21 | 5.18±0.16 |
| $D_3$ | 25.79±0.45 | 6.89±0.21 | 5.28±0.16 |
| $D_4$ | 25.75±0.45 | 6.89±0.21 | 5.20±0.16 |
| $D_5$ | 25.38±0.45 | 6.83±0.21 | 5.31±0.16 |

Means of the same superscript are not significantly different (P>0.05)

### Proximate composition of the *experimental fish carcass*

The carcass composition of the experimental fish after the experiment is shown in Table 4. Fish fed with $D_1$ has the highest protein value while fish fed with $D_3$ has the lowest protein value. Fish fed with $D_1$ has the highest Ash content, while $D_5$ has the lowest value. The moisture content of $D_3$ was the highest while $D_1$ has the lowest Lipid content

**Table 4:** Proximate composition of experimental fish carcass after feeding trial
Mean values of the same superscript are not significantly different on the horizontal row (P>0.05)

| Diets | Samples | | | | |
|---|---|---|---|---|---|
| | %Moisture | %Ash | %Crude fat | %Crude protein | %CHO |
| $D_1$ | 9.96±0.02$^a$ | 15.09±0.04$^d$ | 10.42±0.03$^c$ | 61.25±0.05$^e$ | 3.28±0.01$^b$ |
| $D_2$ | 11.97±0.01$^b$ | 13.62±0.03$^c$ | 15.69±0.05$^e$ | 57.00±0.05$^e$ | 1.72±0.04$^d$ |
| $D_3$ | 12.89±0.02$^a$ | 14.07±0.03$^c$ | 14.29±0.03$^c$ | 55.25±0.05$^e$ | 3.5±0.01$^a$ |
| $D_4$ | 10.59±0.02$^a$ | 14.58±0.03$^c$ | 12.75±0.04$^d$ | 59.25±0.05$^e$ | 2.83±0.04$^d$ |
| $D_5$ | 12.49±0.02$^a$ | 12.53±0.04$^d$ | 12.24±0.04$^d$ | 60.11±0.05$^e$ | 2.49±0.01$^b$ |

**Proximate Composition of Experimental feed**

In Table 5, the protein content in $D_2$ has the lowest value with the highest value in $D_5$. Ash content in $D_4$ (7.31) was the highest and $D_3$ has the lowest value of 5.97. Moisture content of $D_5$ (13.98) was highest with the lowest value in $D_4$ (8.22). Lipid content was highest in $D_2$ (9.68) and lowest in $D_1$ (5.17). Crude fiber content of $D_2$ (12.37) was highest with the lowest value in $D_3$ (10.13). Nitrogen free extract was highest in $D_3$ (26.02) and lowest to be $D_5$ (20.26).

**Table 5:** Proximate composition of experimental feed

| **Diets** | | | **Samples** | | | |
|---|---|---|---|---|---|---|
| | %Moisture | %Ash | %Lipid | %Protein | %Fiber | % NFE |
| **$D_1$** | 11.17 | 7.14 | 5.17 | 40.13 | 10.58 | 25.81 |
| **$D_2$** | 11.78 | 6.00 | 9.68 | 39.5 | 12.37 | 20.75 |
| **$D_3$** | 12.27 | 5.97 | 5.36 | 40.25 | 10.13 | 26.02 |
| **$D_4$** | 8.22 | 7.31 | 7.14 | 41.17 | 10.98 | 25.18 |
| **$D_5$** | 13.98 | 6.98 | 6.78 | 41.33 | 10.67 | 20.26 |

**Growth Performance and Nutrient Utilization of experimental fish**

Table 6 shows the growth and nutrient utilization of *Heterobranchus bidorsalis* juvenile fed with all plant protein diets. The table contains the initial weight gain, specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR), net protein utilization (NPU), final weight gain and mean weight gain of the fish. With the result on the table, the best growth performance and nutrient utilization was recorded in $D_1$ (Control). The specific growth rate (SGR) increased from 0.52 in $D_3$ to 1.17 in $D_1$. Mean weight gain (MWG) and protein efficiency ratio (PER) were optimum (33.99g and 0.86%) respectively at $D_1$ (control).The feed conversion ratio showed a decreasing trend in fish fed $D_4$ (3.11) with the least value in fish fed $D_1$ (2.49).The net protein utilization in fish fed $D_1$ (8.33%) has the highest value while $D_2$ has the lowest value of 7.50%. With these results, the best growth performance and nutrient utilization were recorded in fish fed $D_1$ (control)

**Table 6:** Growth performance and nutrient utilization of experimental fish

| Growth parameters | Diets | | | | |
|---|---|---|---|---|---|
| | **$D_1$**(control) | **$D_2$** | **$D_3$** | **$D_4$** | **$D_5$** |
| Initial mean weight (g) | $26.11\pm0.04^a$ | $26.55\pm0.05^a$ | $25.85\pm0.01^a$ | $26.65\pm0.02^a$ | $26.65\pm0.06^a$ |
| Final Mean weight (g) | $60.10\pm0.04^a$ | $51.00\pm0.04^c$ | $37.40\pm0.04^e$ | $56.00\pm0.05^b$ | $46.00\pm0.04^d$ |
| Mean weight gain (g) | $33.99\pm0.01^a$ | $24.45\pm0.05^c$ | $11.55\pm0.05^e$ | $29.35\pm0.04^b$ | $19.71\pm0.04^d$ |
| Specific growth rate | $1.17\pm0.06^a$ | $0.91\pm0.04^c$ | $0.52\pm0.03^e$ | $1.06\pm0.02^b$ | $0.78\pm0.05^d$ |
| Feed conversion ratio | $2.49\pm0.02^a$ | $2.94\pm0.03^d$ | $2.67\pm0.02^b$ | $3.11\pm0.04^e$ | $2.71\pm0.04^c$ |
| Protein efficiency ratio | $0.86\pm0.06^a$ | $0.62\pm0.04^c$ | $0.29\pm0.05^e$ | $0.72\pm0.01^b$ | $0.47\pm0.03^d$ |
| Net protein utilization | $8.33\pm0.06^a$ | $7.50\pm0.02^e$ | $8.13\pm0.02^b$ | $7.93\pm0.04^c$ | $7.59\pm0.04^d$ |

Mean values on the same row with similar superscript are not significantly different (P>0.05).

## Discussion

All nutrients required for the normal growth of fish must be supplied in formulated diets as the available digestible nutrient. It is a well-known fact that protein is the most important and expensive item of feed that must be provided in the adequate amount to support optimum growth of fish (Zehra and Khan, 2011).The results of this study show that the growth response of *Heterobranchus bidorsalis* juveniles was affected by the plant-protein materials in the diets. Except in the Control diets where the maximum growth of fish was recorded, the growth of fish was impaired in other treatments, indicating poor utilization of feed. From the available nutritional records, *Heterobranchus bidorsalis* prefers animal protein source, mostly fish meal in its diets (Agbebi *et al*, 2009). But fish meal is expensive and in most cases not readily available. Despite the supplementation of test diets with two most limiting amino acids i.e methionine and lysine in the present study, there was no significant improvement in feed utilization as reflected in poor growth performance of the fish. Corn gluten meal is a protein-rich feed ingredient, containing 60 to 70% crude protein. This would have been a potential alternative to fish meal, but corn gluten meal with other plant protein materials in this study were poorly digested by the young *Heterobranchus bidorsalis.* Utilization of all plant- protein feed ingredients by the adult fish of this species may be examined through further research.

## Conclusion and Recommendation

In conclusion, it could be said that all plant-protein meals (corn gluten meal, soya bean meal and groundnut cake) alone are not suitable for optimum growth of *Heterobranchus bidorsalis* juvenile. It is hereby suggested that gradual replacement of fish meal with plant protein materials may give a better results, hence the need for further research. Based on the performance in respect to the growth of fish in this experiment, it is recommended that the inclusion of all plant-proteins ingredients in the diets of *Heterobranchus bidorsalis* juvenile be discouraged. As part of contribution to knowledge, this finding provides useful information to fish farmers especially in the production of *H.bidorsalis* juveniles

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