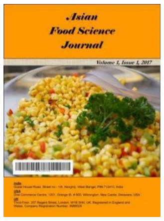

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Replacement of Soyabean Meal with Toasted Lima Beans, (*Phaseolus lunatus*) on Growth and Nutrient Utilization of Clariid Catfish (*Heterobranchus bidorsalis*) Fingerlings

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Authors' contributions

This work was carried out in collaboration between both authors. Author BSA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JO managed the analyses of the study, the field work and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

An experiment was designed and carried out to assess the survival, growth performance and feed utilization (weight gain, feed conversion ratio, protein efficiency ratio, specific growth rate, feed intake and survival) of *Heterobranchus bidorsalis* fingerlings fed graded levels of toasted Lima beans seed (*Phaseolus lunatus*) meal based diets with the aim of establishing the best inclusion level of Lima beans seed meal. One hundred (100) fingerlings with an initial mean weight of 2.5± 0.5g were allotted at random to five treatments in triplicate groups with each treatment tank having five fingerlings and were fed with the compounded diets. The toasted Lima beans seed meal was used to replace soybean meal in the diets in the following proportions: Diet I (0%), diet II (25%), diet III (50%), diet IV (75%) and diet V (100%). At the end of the feeding trials that lasted for 70 days, At the end of the experiment, the Specific growth rate showed no significant difference (P >0.05) among all treatments. Treatment III (3.250) had the highest specific growth rate and Treatment II (2.083) had the lowest value. Relative weight gain was highest in treatment V (20.53)

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with 100% lima beans and lowest in treatment II (16.95) with 25% lima beans diet inclusion level. Treatment I and IV had no significant difference (P >0.05) but these treatments had a significant difference (P<0.05) with treatments II, III and V also treatment II, III, and V are significantly different (P<0.05) from each other. There was no significant different (p>0.05) in the feed conversion ratio of treatment II, III, IV. There was also no significant difference (p> 0.05) in the feed conversion ratio of treatment I and V. Treatment II, III and IV showed a significant difference (P< 0.05) in the feed conversion ratio with treatment I and V. FCR was highest in treatment II (1.383) and lowest in treatment IV (1.162). Treatment V had the highest feed intake with value 3.775. Treatment I, II, III, IV and V had no significant difference (P> 0.05). Treatment I had the lowest feed intake with value (3.246). Protein efficiency ratio showed no significant differences among all treatment. PER was highest in treatment V (6.346) and lowest in treatment III (5.346). The survival rate was slightly different but not as a result of the feed consumed. Based on the findings in this study, it is therefore recommended that 75% inclusion level of Lima beans meal should be adopted in the formulation of feed for *Heterobranchus bidorsalis.*

Keywords: Feed utilization; soyabean; lima beans; Phaseolus lunatus and fish nutrition.

1. INTRODUCTION

Fish is very important to humans due to its very high quality protein as well as the essential amino acids required by the body for growth and maintenance of muscle tissue. Around the world fish protein makes up complete protein sources in many people's Diets. Proteins of high quality, as found in most fresh fish can be used to maintain an active metabolism [1]. In 2007, fish accounted for 15.7% of global animal protein intake and 6.1% of all protein consumed [2]. No doubt, the increasing demand for fish protein can be met when capture fisheries is supplemented by aquaculture. The Nigerian aquaculture industry has grown considerably, contributing to the production of about 20,475 metric tonnes of fish per year in the 1990s to about 85,087 metric tonnes per year in 2007 [3].

Once fish are removed from their natural environment to an artificial one, enough food must be supplied in order to enable them grow. This could be in the form of complete rations, where the artificial diet furnishes all the nutrients required by the fish or supplementary diets, where part of the nutritional needs of fish is supplied by the natural food in the aquatic environment [3]. Nutrient requirement for fish encompasses protein, lipids, carbohydrate, vitamins, and minerals, protein being the major constituents in fish diet presumes that knowledge of its requirement for fish species is essential for the formulation of a balance diet. The main source of protein in fish feed is the animal and plant source origin. Animal proteins are of higher quality than those of plant origin. Animal protein includes fishmeal, meat meal bone meal, and blood meal of which the best protein source for fish feed is fishmeal. Plant protein materials commonly used in fish feed are Soybean meal, groundnut cake and cottonseed cake and the most used and well utilized of these is the soya bean [3].

Soya bean that serves as the most utilizable plant source of protein in feed formulation have become expensive and has to be imported to meet local demands in sub Saharan countries like Nigeria [4]. The fishmeal production has increased the total cost of fish production. [4] reported that the fish used as fishmeal raw materials in year 2000 accounted for about 30 out of 130 tonnes. Inadequate supply of feedstuff, fishmeal in particular which is scarce, expensive and not readily available has hampered aquaculture development [5,6] necessitating the need for the development of fish feed from high quality yet inexpensive product.

Lima bean (*Phaseolus lunatus* L.) is a tropical and subtropical legume cultivated for its edible seeds, a plant protein source and according to [7]. It has been classified as one of the under-utilized legumes in Nigeria. Studies from [8] and [7] showed a resemblance to a common bean in amino acid profile. This class of lesser legume is largely due to a seemingly lack of awareness on its nutritional potentials. Lima bean is widely cultivated in the south-western, south-eastern and the middle belt regions of Nigeria.

2. MATERIALS AND METHODS

2.1 Preparation of Lima Beans and Soyabean Meal

Lima beans *(Phaseolus lunatus)* seeds were purchased from a retail outlet. The matured seeds where dark brown in colour, round, dry and hardy, a total weight of 10 kg was purchased and decoated. The whole seeds were toasted on a well heated pot for ten minutes in the school farm to reduce the effect of toxins and inhibitors such as polyphenols and trypsin inhibitors, after which they were brought down, allowed to cool then milled into fine form.

2.2 Preparation of Experimental Diets

Fishmeal, soybeans cake, corn meal, palm oil as fatty acid and bone meal that were used in the production of the feed were purchased from Liz Enterprises, a private company at Murtala Mohammed Way in Benin City. The vitamin E-gel was purchased from GPS Pharmacy, Third Street, Benin City and the palm oil was obtained from New-Benin market in Benin City. Five isonitrogenous and isocaloric diets were formulated. Diets 1 (control), 2, 3, 4, 5, had soybean meal protein substituted with Lima beans seed meal at 0%, 25%, 50%, 75%, 100% respectively. The composition of the experimental diets is shown in Table 1.

The various ingredients were measured accurately to their required quantity, after which they were homogenously mixed, finely pelleted and dried. The pelleted feed was stored in sealed containers throughout the duration of the experiment.

2.3 Experimental Fish

One hundred *Heterobranchus bidorsalis* fingerlings (mean weight 2.5 ± 0.5 g) were obtained from a hatchery unit of the department farm. They were acclimatized for five days during which they were fed commercial feed.

2.4 Experimental Units

The study was conducted in the wet laboratory, Department of Fisheries, University of Benin, Benin city, Nigeria. Fifteen (15) rectangular plastic tanks, (five (5) treatment in three (3) replicates) measuring (30 cm×36 cm×52 cm) were used. Each tank was filled up to 2/3 of its volume with bore-hole water attached to the laboratory.

2.5 Experimental Procedure

As the period of acclimatization (5 days) came to an end the fishes were weighed in batches of 5 into each of the experimental units replicated three for each treatment. They were fed twice daily to satiation to ensure maximum growth between 8:00 - 9:00 hrs and 15:00 - 16:00 hrs. Feeding was monitored for each unit to ensure that fishes were not underfed or overfed. The experimental units were cleaned by total changing of the water daily and sometimes once in two days. All fishes per replicate were weighed and counted weekly to determine growth and survival, also the weekly weighing of feed was also carried out.

2.6 Parameters Monitored

Data on feed consumed and weight gain were collected weekly for each unit from which the following performance parameters were evaluated.

1. Weight gain (WG) = $W_2 - W_{1\,(g)}$

Where; W_1 = initial weight
W_2 = final weight

2. Feed intake = Initial weight of feed – Final weight of feed

3. Specific growth rate per day (SGR) % = $\frac{\text{Loge W2} - \text{loge W1}}{\text{T2} - \text{T1}} \text{ X } 100$

Where: T_1 and T_2 are time of experiment in days.
W_2 = final weight at T_2
W_1 = initial weight at T_1
Loge = natural logarithm.

4. Relative weight gain (PWG) % = $\frac{\text{Weight Gain}}{\text{Initial Weight}} \text{ X } 100$

5. Food conversion ratio (FCR) = $\frac{\text{Feed Intake(g)}}{\text{Wet Weight Gain(g)}}$ X 100

6. Protein efficiency ratio (PER) = $\frac{\text{Weight Gain (g)}}{\text{Protein Intake}}$ X 100

7. Survival rate % = $\frac{\text{Initial stocked} - \text{mortality}}{\text{Initial stocked}} \text{ X } 100$

2.7 Proximate Analysis of Diets and Fish

A sample of 10 fishes of the initial stock was used for initial analysis, also at the end of the experimental trial some survivals from each treatment were sacrificed and analyzed. Diet samples from the five compounded diets were also collected and a sample of the toasted Lima beans for analysis. They were analyzed using standard methods of the Association of Official Analytical Chemists (AOAC 2000).

2.8 Determination of Moisture Content

This is a measure of the % moisture lost due to drying at a temperature of 105°C, 2 g of the sample was weighed (W1) into pre-weighed beaker (WO) and placed into a hot drying oven at 105C for 3hours. The crucible was removed, cooled in a desiccator and weighed. The process of drying, cooling and weighing were repeated until a constant weight (W2) was obtained. The weight loss due to moisture was obtained by the equation

$$\% \text{ moisture} = \frac{W1-W2}{W1-W0} \text{ X } 100$$

2.9 Determination of Ash Content

This is a measure of the residue remaining after combustion of the dried sample in a furnace at temperature of 60°C for 3 hours. According to James (1995), 1 g of the sample was weighed (W1) into pre-weighed empty crucibles (W0) and placed into a Linton furnace at 60°C for 3 hours. The ash was cooled in a desiccator and weighed (W2). The weight of the ash was determined by the difference between the powdered leave sample, pre-weighed crucible and the ash in the crucible. Percentage ash was obtained by:

$$\% \text{ Ash} = \frac{W2-W1}{W1-W0} \text{ X } 100$$

2.10 Determination of Crude Protein Content

The crude protein of the sample was determined using the micro Kjeldahl method described by AOAC (1990). The principle of this method is based on the transformation of protein and that if the other nitrogen containing organic compounds, other than nitrites and nitrates into ammonium sulphate by acid digestion. The sample (0.5 g) was weighed into a micro Kjeldahl digestion flask of foss automatic digester block system. It was shaken and allowed to stand for some time. One tablet of selenium catalyst with a mixture of 2:1 copper sulphate and potassium sulphate was added followed by the addition of 20 cm^3 concentrated sulphuric acid. The flask was heated on the digestion block at 450°C for 1 hour until digest became clear. An aliquot of the digest (100 cm^3) as transferred into another micro Kjeldahl flask along with 20 cm3 of distilled water and placed in the distilling outlet of the micro Kjeldahl distillation unit. A conical flask containing 20 cm^3 of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution (20 cm^3, 40%) was added to the content in the Kjeldahl flask by opening the funnel stop cock. The distillation start and the heat supplied were regulated to avoid sucking back. When all the available distillate was collected in 5 cm^3 of boric acid mix indicator, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.N of HCL; the end point was obtained when the colour of the distillate changed from green to pink. Crude protein is a measure of the nitrogen in the sample. It was calculated by multiplying the total nitrogen content by a constant, 6.60. This is based on the assumption that, protein contains 16.7%N which includes both true protein and non-protein N and does not make a distinction between available or unavailable protein.

Table 1. Gross composition of the experimental diets (%) on as fed basis

Ingredients	**Diet 1 0% LBM**	**Diet 2 25% LBM**	**Diet 3 50% LBM**	**Diet 4 75% LBM**	**Diet 5 100% LBM**
Fishmeal (65.5% CP	35.40	35.40	35.40	35.40	35.40
SBC (48.0% CP)	40.00	30.00	20.00	10.00	0.00
LBM (36.17% CP)	0.00	10.00	20.00	30.00	40.00
Yellow maize (9.5% CP)	20.00	20.00	20.00	20.00	20.00
Palm oil	8.00	8.00	8.00	8.00	8.00
Bone meal	4.00	4.00	4.00	4.00	4.00
Vitamin premix	0.04	0.04	0.04	0.04	0.04
Vitamin E gel	0.60	0.60	0.60	0.60	0.60
Total	**100**	**100**	**100**	**100**	**100**

LBM= Lima Bean meal, CP= Crude protein

2.11 Determination of Lipid

The lipid content was determined as described by AOAC (1980) using the Soxhlet apparatus for continuous extraction. A clean dry round bottom flask (500ml) containing anti-bumping granules was weighed and about 210cmof petroleum ether (B.P. 60-80°C) was poured into the flask fitted with soxhlet extraction unit. The weighed sample (5 g) was transferred into the thimble which was already fixed into the soxhlet extraction unit. Cold water circulation and the heating mantle was switched on, the heating rate adjusted until the solvent was refluxing at a steady rate. The extraction process was carried out for 8 hours. The solvent was recovered by evaporation and the thimble fitted to the central siphon portion of the extractor of the soxhlet apparatus. The flask and its content were placed in the oven at 70°C for one hour. The flask was cooled in a desiccator and weighed. The flask and the content were then replaced in the oven for 30 minutes after which it was reweighed. This was repeated until the sample was dried to a constant weight. From the weight of the material residue in the receiver flask the percentage lipid content was determined as given below:

$$\% \text{ lipid content} = \frac{\text{Weight of lipid extracted}}{\text{Weight of Sample}} \text{ X } 100$$

2.12 Statistical Analysis

The data obtained from the feeding trials were tested for significant differences using Analysis of Variance (ANOVA) test and the means were separated using Duncan's Multiple Range Test, all at 5% level of significance.

3. RESULTS

Temperature of water ranged from 27-29°C and PH of 7.3- 7.6.

The proximate composition of experimental diet (Table 2) shows that crude fat is highest at treatment III (20.14%) and lowest at treatment V (16.61%), crude fiber content was highest in treatment I (4.12%) and lowest in treatment V (3.54%), moisture content was highest in treatment V (5.55%) and lowest at treatment II (5.22%), Crude protein value was highest in Treatment III (48.51%) and lowest in Treatment I (39.21%), Ash content value was recorded to be highest in Treatment II having a value of (8.27%) and the lowest in Treatment I (6.38%). Nitrogen Free Extract (NFE) was highest in Treatment I (26.98%) and lowest in Treatment III (14.82%).

Table 2. Proximate composition (%) of experimental diets

Proximate composition	Treatment					
	I	II	III	IV	V	Lima beans
Moisture content (%)	5.32	5.22	5.44	5.36	5.55	5.14
Protein content (%)	39.21	41.13	48.51	46.57	45.25	36.17
Ether extract (%)	17.99	18.01	20.14	18.20	16.61	15.28
Crude fibre (%)	4.12	3.49	3.88	3.57	3.54	3.14
Ash (%)	6.38	8.27	7.21	7.65	7.23	7.12
NFE (%)	26.98	23.88	14.82	18.65	21.82	33.15

(Source: Field Survey, 2018)

Table 3. Carcass composition (%) of *Heterobranchus bidorsalis* fingerlings fed varying levels of *phaseolus lunatus* seed meal based diets for 70 days

	Initial carcass	TSF I	TSF II	TSF III	TSF IV	TSF V
Moisture content	5.17	5.31	5.37	5.43	5.16	5.32
Fat	15.23	15.41	16.00	17.23	16.67	15.75
ash	8.11	8.23	7.94	7.18	8.27	7.41
Crude protein	68.25	48.56	53.01	56.50	52.85	56.73
NFE	4.14	22.49	17.68	13.66	17.05	14.79

TSF = Test fish carcass composition (Source: Field Survey, 2018)

Table 4. Growth response and nutrient utilization of *Heterobranchus bidorsalis* fingerling fed *Phaseolus lunatus* seed meal-based diets

Treatment Parameters	I 0%	II 25%	III 50%	IV 75%	V 100%	SEM
Weight gain	2.404	2.317	2.604	2.800	2.921^{NS}	0.377
Feed conversion ratio	1.296^{b}	1.383^{c}	1.321^{c}	1.162^{a}	1.362^{c}	0.1465
Feed intake	3.246	3.367	3.421	3.262	3.775^{NS}	0.344
Relative weight gain	18.95^{c}	16.95^{d}	22.92^{a}	19.79^{c}	20.53^{b}	3.10
Specific growth rate (g)	2.500	2.083	3.250	2.833	2.625^{NS}	0.584
Protein efficiency ratio	6.098	5.596	5.376	5.996	6.346^{NS}	0.837
Survival	100	96.22	98.45	100	100^{NS}	1.20

Mean in each row with the same superscript are not significantly different (P > 0.05) SEM = standard error of mean NS= No Significant Difference (Source: Field Survey, 2018)

The proximate composition of test fish shows that crude protein was highest in test fish fed with diet V having the value 56.73% CP and lowest in test fish fed with diet I (48.56% CP). When compared to the initial carcass (68.25% CP), treatment I, II, III, IV, V had a lower crude protein value. The fat content was highest in fish fed with diet III with the value (17.23%) and lowest in fish fed with diet I (15.41%). Fish fed with diet IV had the highest ash content having the value (8.27%) and lowest in fish fed with diet III having the value (7.18%). Test fish fed with diet I had the highest Nitrogen Free Extract (NFE) value of 22.49% and the lowest value in test fish fed with diet III 13.66%.

The growth response and nutrient utilization of test fish is presented at Table 4. At all levels of substitution, there was an increase in weight gain, the highest weight gain was 2.921 g recorded in fish fed with diet containing 100% inclusion level of lima beans meal. This treatment was not significantly different (p> 0.05) from all other treatment (I, II, III and IV). The lowest weight gain was recorded in fish fed 25% inclusion level of lima beans diet

Relative weight gain was highest in treatment V (20.53) with 100% lima beans and lowest in treatment II (16.95) with 25% lima beans diet inclusion level. Treatment I and IV had no significant difference (P >0.05) but these treatments had a significant difference (P<0.05) with treatments II, III and V also treatment II, III, and V are significantly different (P<0.05) from each other. Treatment V had the highest feed intake with value 3.775. Treatment I, II, III, IV and V had no significant difference (P> 0.05). Treatment I had the lowest feed intake with value (3.246). Specific growth rate in treatment II, III, IV and V showed significant no difference (P <0.05) from treatment I. Treatment III (3.250) had the highest specific growth rate and treatment II (2.083) have the lowest value. There was no significant different (p>0.05) in the feed conversion ratio of treatment II, III, IV. There was also no significant difference (p> 0.05) in the feed conversion ratio of treatment I and V. Feed conversion ratio in Treatment II, III and IV was significantly different (P< 0.05) from the value of the feed conversion ratio in treatment I and V. FCR was highest in treatment II (1.383) and lowest in treatment IV (1.162). Protein efficiency ratio showed no significant difference (p>0.5) across all treatments (I, II, III, IV, V). PER was highest in treatment V (6.346) and lowest in treatment III (5.346).

4. DISCUSSION

4.1 Proximate Composition of Lima Beans

The crude protein content and ether extract of toasted lima beans seed was recorded to be 36.17% CP and 15.28% respectively; these values are higher than the 31.27% CP and 10.12% reported by [9]. This indicates that there are factors which affect the crude protein and fat content such as the processing methods. [10] reported that cooking of mucuna bean reduces the crude protein content of raw Nigeria and Brazilian seeds by 5.3% and 6.5% respectively. [11] also noted that raw lima bean had a higher protein, lipid and ash content when compared with soaked, autoclaved and toasted lima beans seed. The usual approach to formulating Diets for simple-stomach animals is to use ingredients that will maintain Dietary fiber levels below acceptable maximum levels. These levels would be in the range of 3- 6% crude fiber for catfish Diets [12]. Lima beans had 3.14% of crude fibre as shown in this study and this must have influence the value obtained in the experimental diet to be in the appropriate range

4.2 Growth

The experimental fish within all the treatments showed great increase in weight, which indicates that the fishes were able to convert feed protein to extra muscles. Weight gain and specific growth rate are usually considered as the most important measurement of productivity of Diets [13]. From this experimental study, the result showed that treatment III (50% LBM) had a better specific growth rate when compared to other experimental diet including the control (TRT I, 0% LBM). This is followed by treatment IV (75% LBM). This is slightly correlating with the study carried out by [9] of methionine supplemented toasted lima beans fed to oreochromis niloticus in which growth rate was found to be highest for diet containing the same inclusion level of Lima bean meal. The least growth was observed in treatment II (25% LBM). This is also in Harmony with the assertion of [9] in their related study as stated above. The weight gain was highest with fish in treatment V (100% LBM) and lowest in treatment II (25% LBM). The low level of crude fibre in lima beans as shown in this study must have improve the palatability of the diet hence having a favourable effect on the digestibility which obviously had influenced the weight gain. The low weight gain experienced in treatment II could have been as a result of the imbalance in the plant protein source. It was documented [14] that some levels of amino acids in cracked soybean seed decreased after heat treatment. The study showed no significant difference (P<0.05) between the growth performance (percentage weight gain and specific growth rate) of the fingerlings fed the compounded Lima beans meal substituted diets (Treatment II, III,IV and V) and those fed the conventional soyabean meal diet (Treatment I). This can be attributed to proper utilization of the LBM, the suitability of the processing method used to adequately eliminate the anti-nutritional factors. This corroborates with the finding of [15] who reported that reductions in anti-nutrients by different processing technique result in better palatability and growth in fish. Heat treatment has been shown to improve dietary utilization in legumes [16,17].

4.3 Nutrient Utilization

Feed utilization expressed as FCR is known to be affected by body weight, ration and size and temperature [18]. The lower the food conversion ratio indicates higher protein conversion efficiency thereby resulting in better growth. [19] and [20] documented that the lower the FCR, the better the feed utilization by the fish. From this study, the feed conversion ratio of (1.162) and (1.321) obtained by fingerlings fed diet containing Lima 75% (Trt IV) and Lima 50% (Trt II) respectively are lower compare to others except from the control diet (0% lima) which is yet higher than diet Treatment IV but lower than treatment III. The result was in contrast to the study carried by [8] in which the lowest value of FCR was reported in Treatment II (25% Lima) however in their experimental study, Lima beans was been substituted with fish meal.

Protein Efficiency Ratio (PER) is known to be regulated by the non-protein energy input of the Diet and is a good measure of the protein-sparing effect of lipid and/or carbohydrate [21]. The PER of the experimental fish obtained in this study exhibited no significant differences P>0.05 in all treatments. The PER values increased among the experimental fish with the highest recorded in treatment V (Lima beans 100%). Similar observations were made by [22].

All the experimental diets were accepted by the experimental fish indicating that the incorporation of LBM in fish diets did not have adverse effect on the palatability of the experimental diets. It has been noted that cultured fish in artificial enclosures such as cages depend solely on the nutrient from the feed for growth with little or no contribution from natural food. This implies that the general increase in weight of trial fish was an indication that all the diets met a part or the whole nutrient requirement for growth in *Hetrobranchus bidorsalis* fingerlings.

5. CONCLUSION

The diet fed to H. *bidorsalis* had significant effect on their growth and nutrient utilization, the result obtained from this study showed that among the diet which contained Lima beans meal, diet IV (75% lima) had the best performance level in experimental fish although the highest weight gain was observed in treatment V, but the value is slightly higher than what was observed in treatment IV which had a better a better Specific growth rate and feed conversion ratio.

This study has demonstrated that Lima beans have the potential to replace soya beans; this would considerably reduce the expenditure on soya beans without compromising growth performance and feed utilization of the African catfish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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