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Effect of Substitution of Soyabean with Dietary Toasted Lima Beans, (Phaseolus Lunatus) on Growth and Nutrient Utilization of Clariid Catfish (Clarias Gariepinus) Fingerlings

***Aliu B.S. and Okoh A.**
***Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, P.M.B. 1154, Benin City, Nigeria.**
E-mail: bayo.aliu@uniben.edu
+2348055314843
***Corresponding author**

Abstract
A feeding trial was conducted to study the effects of supplemented toasted lima bean (*P.lunatus*) diets on the growth performance and utilization of *Clarias gariepinus* fingerlings. TLBM was used to replace soybean meal at 0% (L0), 25% (L25), 50% (L50), 75% (L75) and 100% (L100) producing five diets. Diets were fed to triplicate groups of *C. gariepinus* daily to satiation. One hundred (100) fingerlings with an initial mean weight of 2.0g were allotted at random to five treatments in triplicate groups with each treatment tank having five fingerlings and were fed with isocaloric and isonitrogenous diets. Results indicated that the mean weight gain was significantly ($P<0.05$) higher in fish fed diet 2(6.34) and diet 5(5.02) than all other treatments. Feed Conversion Ratio (FCR) was significantly higher ($p<0.05$) in diet 2(1.20), diet 1(1.56), diet 5(1.520 and diet 4(1.64). Specific growth rate (SGR) and protein efficiency ratio (PER) showed no significant difference ($P>0.05$) as a result of the treatments. The specific growth rate was significantly higher in diet II (4.37) while diet V has the least specific growth rate 2.53. Summary of findings highlights that soybean meal can be replaced up to 25% and 100% of Toasted lima bean meal in the diets of African catfish, *C. gariepinus* fingerlings without compromising the growth and carcass composition.

Keywords: feed utilization, Soyabean, Lima beans, *Phaseolus lunatus* and fish nutrition

Introduction.

Feed is, without question, the single most expensive input in intensive fish culture (Agbebi *et al*, 2009) especially for catfish which needs a high protein diet (as it is cannibalistic). This is due to the cost of raw materials, which are usually imported and are necessary for its production (fish meal and wheat offal or corn products). Research has shown that most imported feedstuff can be replaced by locally available feedstuff (Faruque *et al.*, 2010). This, therefore, entails the production of fish feed from locally available materials using local technology in order to reduce the cost and improve availability of feed to farms.

Fish meal is currently one of the major sources of animal protein used in diets for marine shrimp (Sá *et al*., 2013). Protein is the most expensive ingredient in the diet, and its quality is a very important nutritional aspect in aquaculture (Gomez *et al*., 2008). Much of the raw material Used in the production of this input comes from pelagic fishing (Tacon and Metian, 2008). Plant proteins are cheap and readily available, but they have some limitations that make them Unsuitable for direct incorporation into animal feeds (Aletor and Fetuga, 1984). Leguminous seeds such as soybean, faba bean, pea, mung bean, cowpea, kidney bean, pigeon bean and lima bean constitute an important and inexpensive dietary source for many people particularly in the developing countries (Al-Abdalall, 2010).

Substitution of fishmeal with plant proteins has been reported to reduce growth rate especially of carnivorous and omnivorous species (Hansen *et al*. 2007). Inclusion of plant ingredients like sunflower (Helianthus annuus) cake (Nyina-Wamwiza *et al*., 2007), cotton (*Gossypium* sp.) seed meal (Imorou-Toko *et al*., 2008), soybean, corn, wheat (*Triticum aestivum*) middling's and cotton seed meal (Li *et al*., 2010) in fish feed have been reported to reduce fish growth rate in comparison to fishmeal. Similarly plant ingredient like leaf meal of *Amaranthus spinosus* (Adewolu and Adamson, 2011), was found to reduce fish growth rate compared to fishmeal diets. The reduced growth rate of fish fed with plant proteins is due to several reasons such as anti-nutritional factors (ANFs) inherent in most plant ingredients (Francis *et al*., 2001), imbalanced amino acid composition or poor digestibility (Lech and Reigh, 2012). The replacement of fishmeal with plant proteins may also reduce feed palatability and consequently reduce feed intake (Tiril *et al*., 2008). Orisasona *et al*. (2014) observed that boiled Lima Bean Meal (BLBM) successfully replace 50% SBM in the diets of *C. gariepinus*, though; boiling only slightly reduced the phytate content of BLBM. Orisasona and Ajani (2015), successfully deduced that phytase supplementation in fish feed will enhance feed digestibility, thus increasing nutrient availability which will in turn increase growth of cultured fish. The dearth of information on Lima beans in lieu with their optimum nutritional and functional properties has made this potentially beneficial legume under-utilized.

Materials and Methods

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 10kg 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.

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

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

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.

Experimental Fish

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

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 (30cm×36cm×52cm) were used. Each tank was filled up to 2/3 of its volume with bore-hole water attached to the laboratory.

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 - 9:00hrs and 15:00 - 16:00hrs. 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.

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) % = $\frac{\text{Weight Gain}}{\text{Initial Weight}}$ 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 % = $\frac{\text{Initial stocked} - \text{mortality}}{\text{Initial stocked}}$ X 100

Statistical Analysis

All analyzed data were tested for significant differences using analysis of variance (ANOVA) test and the means were compared using Genstat 2012 version, all at 5% level of significance.

RESULTS

Growth and Feed Utilization Parameters

Table 2: Growth performance and feed utilization of *Clariid* catfish (*Clariasgariepinus*) to Toasted Lima Bean Meal (LBM) based diet.

Growth parameter	TRT 1 (0% LBM)	TRT 2 (25% LBM)	TRT 3 (50% LBM	TRT 4 75% LBM	TRT 5 100% LBM	SED
Weight Gain(g)	4.09^{b}	6.34^{a}	3.17^{b}	3.81^{b}	5.02^{ab}	0.93s
Specific Growth Rate (%/day)	3.14	4.37	2.59	2.73	2.53^{NS}	1.04
Relative Weight Gain(%)	19.26	26.68	21.54	19.43	19.90^{NS}	4.43
Protein Efficeincy Ratio	3.47	5.04	2.66	2.22	2.96^{NS}	1.31
Feed Intake(g)	4.44	5.16	4.48	4.53	5.58^{NS}	0.76
Feed Conversion Ratio	1.56^{ab}	1.20^{a}	2.31^{b}	1.64^{ab}	1.52^{ab}	0.40
Net Protein Utilisation(%)	67.93^{a}	10.35^{b}	53.03^{a}	16.00^{b}	11.79^{b}	8.52

Mean values with the same superscript on the same row are not significantly different (P>0.05). NS- Non significance

The results from Table 2 showed that weight gain of *Clariasgarepinus* fingerlings after 10 weeks (70 days) was significantly higher (p< 0.05) in diet 2(6.34) and diet 5(5.02) than all other treatments. However diet 1(4.09), diet 4(3.81) and diet 3(3.17) did not significantly differ (p>0.05), while diet 3(3.17) recorded the least value amongst all treatment. The specific growth rate for diet I,II,III, IV, Vwas not significantly different (p>0.05) from each other. However, the specific growth rate was significantly higher in diet II (4.37) while diet V has the least specific growth rate 2.53. There was no significant difference (P>0.05) in the relative weight gain for all the treatments. However, The Relative Weight gain for diet 2 (26.68) was significantly superior (P<0.05) than all the other treatments while Diet 1 (19.26) recorded the least value. The protein efficiency ratio was not significantly different (P>0.05) in all the treatment. However the protein efficiency ratio was highest in fish fed diet II (5.04), while diet IV (2.22) recorded the least protein efficiency ratio. The feed intake of the diet in ascending order were; 4.44, 4.48, 4.53, 5.16, 5.58, for diet 1, diet 3, diet 4, diet 2, and diet 5 respectively. There was no significant difference (p>0.05) in the feed intake in the various diets. Feed conversion ratio was best in diet 2 (1.20) while diet 3(2.31) has the highest feed conversion ratio. However, the feed conversion ratio was significantly higher (p<0.05) in diet 2(1.20), diet 1(1.56), diet 5(1.520 and diet 4(1.64). While diet 3(2.31) recorded the least feed conversion ratio. Net protein utilization in diet 1and diet 3 were not significantly different (p>0.05) from each other. Diet 1(67.93) however was significantly higher (p<0.05) than other diets while diet 2(10.35) was significantly lower (p<0.05).

Table 3: Carcass composition (%) of *Clarias gariepinus* fingerlings fed varying levels of Lima Bean Meal (*phaseolus lunatus)* based diet for 70 days

DIETS/PROXIMATE COMPOSITION	Crude protein	Fat	Ash	MC	NFE
Fish (Initial) carcass	65.92	18.26	7.75	5.34	8.15
TSF I (Control)	49.00	13.69	8.34	4.46	25.18
TSF II	66.50	13.69	8.34	5.24	1.22
TSF III	54.83	13.69	8.34	5.12	15.06
TSF IV	68.25	15.65	8.74	5.16	2.20
TSF V	67.08	16.20	7.96	4.33	4.16

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

Proximate composition of test fish (Table 3) shows that Crude protein level was irregular been highest in fish fed the test diets with 68.25% in diet IV and lowest at 49.00% in diet I. The fat content was highest in the initial fish carcass18.26%. Test fish fed with diet I, II and III recorded the same value 13.69% which recorded the lowest value. Ash content of test fish was highest in fish fed with diet IV at8.74% and similar value was recorded in diet I, II and III at 8.34 while the lowest is in the initial fish carcass with value of 7.75. Moisture content was highest in the initial fish carcass at 5.34% and lowest in fish fed with diets V at 4.33%. Carcass fed with diet IV had the highest crude protein value (68.25% CP) and carcass fed with diet I had the lowest value (49.00% CP). When compared with the initial value (65.92% CP), treatments II, and V increased with 66.50% CP, and 67.08% CP respectively, with reduced CP value recorded in diet III at 54.83 % CP.

Table4: Gross Proximate Composition (%) of Experimental Diets

TREATMENT/Proximate Composition	Crude Protein	Fat	Ash	MC	Crude fibre	NFE
1	18.08	18.24	6.35	5.15	3.43	48.74
II	38.50	19.21	7.14	6.27	4.11	24.77
III	20.42	17.34	8.25	6.05	3.54	44.41
IV	56.58	18.33	7.13	6.37	3.43	8.15
V	35.00	16.14	7.24	5.24	3.55	32.82
LBM	36.17	15.28	7.12	5.14	3.14	33.15

The proximate composition of experimental diet (Table 4) shows that crude fat is highest at treatment II (19.21%) and lowest at treatment V (16.14%), crude fiber content was highest in treatmentII(4.11%) similar in treatment I and IV (3.43) which also has the lowest percentage. treatment, moisture content was highest in treatment IV (6.37%) and lowest at treatment I (5.15%), Crude protein value was highest in Treatment IV (56.58%) and lowest in treatment I (18.08%), Ash content value was recorded to be highest in treatment III (8.25%) and the lowest in Treatment I (6.35%). Nitrogen Free Extract (NFE) was highest in Treatment I(48.74%) and lowest in treatment IV (8.15%).

Discussion

Proximate Composition of Lima Bean

The results of the proximate composition of the experimental diet showed that the crude protein of the toasted Lima Bean meal (LBM) was 36.17% which is higher than the 22.50% and 22.92% crude protein recorded by Falaye *et al.,* (2014) and Akinmutimi and Ezea, (2006).This implies that some factors are responsible for the these differences and among these factors are processing methods Ndidi *et al*., (2014) and Adegunwa *et al*., (2012), variety, as well as environmental conditions. . Processing has also been found to reduce protein content but increase protein digestibility. The complete elimination of trypsin inhibitor by toasting agreed with the report of Ologhobo (1992), where heat treatments such as toasting, autoclaving and cooking were shown to destroy heat-labile anti-nutritional factors.

Martin-Cabrejas*et al*., (2009) and Rehmon and Shah (2005) found that protein content reduced while protein digestibility increased with soaking and cooking in chickpea and lentils respectively. The result also showed low moisture content which fell within the recommended range of 0 –13.5% as reported by James (1995). The moisture content of toasted lima beans is 5.14% which is lower than 7.50% and 7.10% that was recorded by Elizabeth *et al.,*(2018) in raw and toasted lima beans respectively. Moisture content in a food is an index of its water activity, foods with high moisture content are prone to quick spoilage due to microbial activities (Aruah *et al*., 2012). Fish fed compounded diets actively grew efficiently without external sign of nutritional deficiencies. However, growth performance in terms of weight gain of fish fed LBM diets at various inclusion level up to 100% replacement were significantly higher in diet II and V,diet III and diet IV was not significantly different with diet 1 having the least weight gain.

Feed Intake

There was no significant (P>0.05) difference in feed intake of the experimental fish fed diets containing the toasted lima bean meal: this result is similar to that indicated by Akinmutimi and Ezea, (2006) who reported that there was no significant (P>0.05) difference in feed intake among the rabbits fed diets containing the toasted lima bean meal. The feed intake of the diet in ascending order were; 4.44, 4.48, 4.53, 5.16, 5.58, for diet 1, diet 3, diet 4, diet 2, and diet 5 respectively. This could be attributed to the effect of a corresponding increase in residual antinutritional factors (Udedibie and Carlini, 1998). For example, tannin has been reported to reduce feed intake by causing poor palatability of the diet containing it (Aletor and Fasuyi, 1997). The non-significant decrease in feed intake shows the superiority of toasted lima bean meal to the raw one which has been reported to effect poor feed intake at 30% dietary level of inclusion (Arijeniwa and Igene, 2002).

Mean Weight Gain

Experimental fish also showed a positive response in terms of mean weight gain with treatment II (6.34) and treatment V(5.02), been highest, treatment I (4.09), III (3.17) and IV (3.89) showed similar response. Inclusion level at 25% (treatment II) and 100% (treatment V) produced best result, this finding correlate with Orisasona *et al.*(2014) who reported that 25% Boiled Lima Bean substitution of *soyabean* in diets gave the best growth performance. This indicates that toasted LBM can be use as a sole protein source for *C. gariepinus*. However, this does not correlates with Aliu and Okolie (2005), who reported that 40% inclusion levels produced highest mean weight gain. Reduction in weight due to hydrogen cyanide has been reported by Akinmutimi (2004). Earlier on, Aletor and Fasuyi (1997) explained that its detoxification requires organic sulphur donors inform of methionine and cysteine, thereby precipitating, methionine deficiency in an otherwise balanced diet. It is this deficiency that causes poor weight gain (growth). Also tannin has been reported to bind with the proteins of saliva and mucosa membrane (D'mello and Devandra, 1995), forming complexes that are not readily digestible. The cumulative effect is poor growth.

Specific Weight Gain (SWG)

There was no significant difference in the Specific Growth Rate of the experimental fish; this result is similar to that indicated by Oso *et al*., (2013) who reported that In substituting for fishmeal, have showed no significant difference (P<0.05) between the growth performance (weight gain, % weight gain and specific growth rate) of the fingerlings fed the compounded Bambara nut diets up to 75% supplement and those fed the conventional fish meal, this can be attributed to proper utilization of the compounded BGM, hence, it was able to effect specific growth rate comparable to fish meal.

Feed Conversion Ratio (FCR)

There was no significant difference (P>0.05) in the feed conversion ratio of treatments I, II, IV and V indicating that food was converted to flesh at different rate, treatment III was significantly different from all the treatment. However, The feed Conversion Ratio (FCR) of this experiment showed highest value in treatment III with inclusion level of 50%, followed by treatment IV (75% inclusion), treatment V (100% inclusion), treatment I (0% inclusion), while treatment II with an inclusion level of 25% has the lowest FCR which tallied with Aliu and Okolie (2005), that recorded that the Bambara groundnut feed gave lower feed conversion levels. This study however does not correlate with the report of Uchechukwu *et al.,* (2014), who reported that increasing level of soybean recorded decrease in FCR. This study also correlate with the report of Hassan *et al.,* (2015) who recorded the best (1.00±0.00) feed conversion ratio for *C. gariepinus* when replacing soybean with Boabab seed at 10%. The lower the FCR, the better the feed utilization by the fish (Adikwu, 2003; Jabeen*et al*., 2004)

Protein Efficiency Rato (PER)

The protein efficiency ratio was not significantly different in all the treatment. This confirm to the study of Orisasona *et al*. (2014) who reported that the Apparent digestibility coefficient (ADC) of protein was significantly reduced with increasing BLBM inclusion from 75.27±3.58% in L0 to 64.46±4.75% in L100. According to Tibbetts *et al.,* (2005). Protein efficiency ratio is known to be regulated by the non- protein energy input of the diet and this is a good measure of the protein-sparing effect of lipids and/or carbohydrates.

The result of the study by Orisasona *et al*. (2014) on Effect of replacement of soyabean with lima bean (*Phaseolus lunatus*) meal on growth and hematological parameters of *Clarias gariepinus* juveniles burchell, 1822) showed that soybean meal can be replaced with up to 50% of boiled lima bean meal in the diets of African catfish, *C. gariepinus* fingerlings which suggest that lima bean has the potential of replacing or competing with soya beans in fish feed formulation. Orisasona and Ajani (2015) also reported that phytase supplementation in fish feed will enhance feed digestibility, thus increasing nutrient availability which will in turn increase growth of cultured fish. The effective utilization of phosphorus by fish fed phytase diets will surely reduce the level of phosphorus released as fecal wastes into the environment, thereby reducing eutrophication in ponds and adjoining water bodies. Banyigyi *et al*., (2001) have suggested that extension of the duration of heat processing or employment of feed processing methods such as boiling or fermentation may enhance better growth and feed utilization.

Conclusion

The result obtained from this study showed that Diet II (25% inclusion) had the best response but this was not significantly different from Diet V with 100% inclusion level which performed best among the other Diet that had TLB present in it. However the inclusion of TLB in the diet of *Clarias gariepinus* had no adverse effect on growth rate. The Feed Conversion Ratio (FCR) was low with Diet II (25% inclusion) best followed by Diet IV with 100% inclusion level and the cost of feed per kg weight was reduced. Therefore, from the study carried out, the recommended level of TLB is 25% and 100% respectively for catfish (*C. gariepinus*) since they performed better.

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