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Effect of dietary replacement of soyabean meal with toasted lima bean meal on the growth and nutrient utilization of heteroclarias juveniles

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Abstract
This study examined the substitution of Soya bean meal with Lima beans meal (LBM) in practical diets for Clariid Hybrid catfish (*Heteroclarias*). Six iso-nitrogenous and iso-caloric diets with a crude protein level of 40% and energy level of 15% were formulated and fed to *Heteroclarias* juveniles. Treatment 1, 2, 3, 4, 5, 6 had soybean meal protein substituted with *P. lunatus* seed meal at 0% (control), 20%, 40%, 60%, 80% and 100% respectively.
The result obtained from the study showed that the highest weight gain was recorded in treatment 4(2.28g) that was fed with diet containing 60% Lima beans meal, while Treatment 6 had the lowest weight gain value of 1.58g. There was no significant difference ($P>0.05$) among the six treatments in terms of feed intake. However, Treatment 6 had the highest feed intake of 4.15g, while the lowest value of feed intake (3.48g) was recorded in treatment 3. Treatment 4 had the best feed conversion ratio (FCR) of 2.20, while the worst FCR value (3.27) was recorded in treatment 5. There was a gradual increase in terms of FCR across the various treatment from Treatment 1 to treatment 5. The highest relative weight gain (RWG) was recorded in treatment 4 (28.85), this treatment was not significantly different ($P>0.05$) from treatment 3 (14.71) but was significantly different ($P<0.05$) from the rest of the treatments. The values gotten indicate that inclusion levels did not affect the survival of the fish. In conclusion, result obtained from this study showed that among the diets with toasted Lima bean meal, Treatment 4 with 60% inclusion rate had the best performance and feed utilization value. This further demonstrate that toasted Lima beans can partially replace Soya bean meal in the diet of *Heteroclarias*.

Keywords: *Heteroclarias*, lima bean, soyabean, fish nutrition

Introduction
Fish is one of the most important protein foods available in Nigeria [1]. It is usually the main source of animal protein in the diet of low, middle level income groups and the rich [2]. The fish species that are commonly cultured in Nigeria are *Clarias gariepinus*, *Heterobranchus bidorsalis*, Heteroclarias, *Oreochromis niloticus* and *Cyprinus carpio* [3]. African mud catfish (*Clarias gariepinus*) is the most cultured fish species due to its high growth rate, large size, good flesh quality, tolerance to poor water quality, high feed responsiveness, economic viability, high cultural acceptance, ability to withstand high stocking density resistance to diseases, good taste and high consumer preference. Many fish farmer's culture catfish as they have a higher market value (2-3 times) than that of other species.
Feed is, the single most expensive input in intensive fish culture [4] especially for catfish which needs a high protein diet. This is due to the cost of raw materials, particularly fishmeal and micronutrients which are usually imported. Research has shown that most imported feedstuff can be replaced by locally available feedstuff to reduce cost [5]. There is, therefore, the need for production of fish feed from locally available materials using local technology in order to reduce the cost and improve fish feed availability.
Like other grain legumes, lima beans are relatively rich in protein. They contain about 25% protein in the DM, a value comparable to that of peas and cowpeas (*Vigna unguiculata*), but variability is remarkably high (from 19 to 28% DM). Starch content is also high, about 40% DM, though lower than the starch content of peas and cowpeas. Lima beans are low in cell wall constituents and low in fat (less than 1.5% DM). The main limitation of lima beans in animal nutrition is the presence of anti-nutritional factors, their elimination requiring processing.

During germination, there is an increase in phytase activity that results in a decrease in phytate content, which could have a positive impact on poultry and ruminant nutrition, and on the environment [6].

Soybean is a major feedstuff, commonly used as a protein source in fish diets. Due to its competitive use in livestock and pharmaceutical industry and for human consumption its cost has increased beyond the reach of fish farms. There is therefore the need for research into non-conventional protein ingredients such as toasted lima beans (*Phaseolus lunatus*) that can replace soybean without compromising fish growth and health.

Materials and methods

Study Area: The study was carried out in the Department of Aquaculture and Fisheries experimental farm, University of Benin, Edo State, Nigeria located on latitude $6^0 36^0$N and Longitude $06^0 19^0$E.

Preparation of Lima Bean and Soyabean Meal: *Phaseolus lunatus* seeds was purchased from a retail outlet in New Benin Market. The seeds were sundried for two days to ensure all seeds were dried uniformly. A pot was placed on fire and heated up with low heat for 2 minutes before the seeds were introduced into the pot. The seed was stirred continuously for 30 minutes at a temperature 55ºC till the seed coat became reddish brown in color and emitting a sweet aroma. They were then removed and allowed to cool before milling to flour. It was there-after sieved to remove the skin of the seeds. Fresh soyabean seed was purchased from a retail outlet in Benin City and poured into a preheated pot and toasted in a similar manner as was carried out with the *Phaseolus lunatus* seeds and milled.

Preparation of Experimental Diets: Fishmeal, maize, bone meal, vitamin premix and vitamin E-gel were bought from a retail outlet in Benin City, Nigeria. Six iso nitrogenous and iso caloric diets with a crude protein level of 40% was formulated. Diets 1 (control), 2, 3, 4, 5, 6 had soybean meal protein substituted with toasted lima bean meal at 0%, 20%, 40%, 60%, 80% and 100% respectively. The composition of the experimental diets is shown in Table 1.

Experimental Design: The experiment was laid out as a complete Randomized Design (CRD) with six treatments and three replicates. Treatment1 (control), 2, 3, 4, 5, 6 had soybean meal protein substituted with toasted lima bean meal at 0%, 20%, 40%, 60%, 80% and 100% respectively.

Table 1: Gross Composition of the Experimental Diets (%) on as fed basis

Ingredients	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	T_5 (80%)	T_6 (100%)
Fishmeal (65.5% CP	30.00	30.00	30.00	30.00	30.00	30.00
SBC (48.0% CP)	40.00	32.00	24.00	16.00	8.00	0.00
Lima bean meal(37.5CP)	0.00	8.00	16.00	24.00	32.00	40.00
Yellow maize (9.5% CP)	15.00	15.00	15.00	15.00	15.00	15.00
Palm oil	8.00	8.00	8.00	8.00	8.00	8.00
Bone meal	6.00	6.00	6.00	6.00	6.00	6.00
Vitamin premix	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin E gel	0.60	0.60	0.60	0.60	0.60	0.60
Total	100	100	100	100	100	100

LBM= Lima Bean meal, CP= Crude protein

The required quantity of ingredient for each of the diet (Table 1) was weighed and mixed into a homogenous mixture with the exception of corn meal. The component of corn meal in each diet was gelatinized (boiled in water to form gel), and used as binder. They were all made into pellets with a pelleting machine in the Department of Fisheries experimental farm at the University of Benin. The diets were then dried using Altona smoking kiln at 105ºC in the Department of Fisheries Experimental Farm and then stored in air-tight containers throughout the experimental period.

Experimental Fish: One hundred and fifty (150) *Heteroclarias* juveniles of mean weight 10± 0.5g was obtained from the hatchery unit of the Department of Fisheries experimental farm, University of Benin. They were acclimatized for 7 days in the laboratory during which they were fed Coppens commercial feeds.

Experimental Units: Eighteen rectangular (18) plastic tanks (six (6) treatment in three (3) replicates) measuring (30cm×36cm×52cm) was used. The experiment was carried out at the Department of Fisheries wet laboratory, University of Benin. Each tank was filled with water up to 2/3 of its volume with bore-hole water attached to the laboratory.

Experimental Procedure: At the end of acclimatization, the fishes were weighed in batches of five randomly stocked 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. All fish per treatment was weighed and counted weekly to determine growth and survival.

Parameters Monitored

1. Weight Gain (WG) = $W_2 - W_{1\,(g)}$ (Bagenal, 1978)

Where
W_1 = initial weight
W_2 = final weight

2. Feed Intake = initial weight of feed – final weight of feed. (Bagenal, 1978)
3. Specific Growth Rate (SGR) % $= \frac{\text{Loge W2} - \text{loge W1}}{\text{T2} - \text{T1}} \text{ X } 100$. (Bagenal, 1978)

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 (RWG) % $= \frac{\text{Weight Gain}}{\text{Initial Weight}} \text{ X } 100$. (Bagenal, 1978)
5. Survival Rate % $= \frac{\text{Initial stocked} - \text{mortality}}{\text{Initial stocked}} \text{X } 100$. (Bagenal, 1978)

Absolute Growth Rate (AGR) $=\frac{increase\ in\ weight}{time}$. (Bagenal, 1978)

Food Conversion Ratio (FCR) %$=\frac{\text{Feed Intake(g)}}{\text{Wet Weight Gain(g)}}$. (Bagenal, 1978)

Proximate Analysis: The various diets and Toasted Lima beans were analysed for their proximate composition which include their moisture content, nitrogen, ether extract, crude fibre and nitrogen-free extract (NFE) according to the procedures of Association of Official Analytical Chemists [7].

Statistical Analysis: The data obtained from the feeding trials was analysed using the computer software Genstat Version 8.1 (2005). Completely randomized design in a one-way ANOVA was used to calculate the mean. The differences in mean was compared using Duncan's multiple range tests at 5% significant level.

Results

The result of the study is shown in table 2. Highest weight gain was recorded in treatment 4 (2.28) that was fed with diet containing 60% Lima beans meal. This treatment was not significantly different (P >0.05) from treatment 3 (2.20). Diet containing 100% lima beans meal had the lowest weight gain value of 1.58g. Generally, weight gain increased with increasing rate of substitution up to 60% substitution rate (Treatment 4) after which the weight dropped with increase in substitution. There was no significant difference (P>0.05) among the six treatments in terms of feed intake meaning that feed was consumed at the same levels of intensification within each treatment. However, Treatment 6 had the highest feed intake of 4.15g, this was followed by treatment 5 with a feed intake value of 3.82g while the lowest value of feed intake (3.48g) was recorded in treatment 3.

The feed conversion ratio showed no significant difference (P>0.05) among the six treatments. However, Treatment 4 had the best feed conversion ratio of 2.20, this was followed by treatment 3 with a feed conversion ratio of 2.52 while the worst feed conversion ratio (3.27) was recorded in treatment 5. There was a gradual increase in terms of feed conversion ratio across the various treatment from Treatment 1 to treatment 5. However, feed conversion ratio value decreased from 3.27 in treatment 5 to 3.06 in treatment 6.

The relative weight gains of fish fed various diets data evaluated displayed a notable trend with various substitution levels. The highest relative weight gain was recorded in treatment 4 (28.85) that was fed with diet containing 60% Lima beans meal. This treatment was not significantly different (P>0.05) from treatment 3 (14.71) but was significantly different (P<0.05) from the rest of the treatments. Diet containing 80% lima beans meal had the lowest relative weight gain value of 4.80.

The highest specific growth rate was recorded in treatment 4 (14.41) that was fed with diet containing 60% Lima beans meal. Diet containing 100% lima beans meal had the lowest specific growth rate value of 6.84. The specific growth rate increased with increasing rate of substitution up to 60% substitution rate (Treatment 4) after which it dropped with the increase in substitution.

Table 2: Growth and nutrient Utilization of Heteroclarias juveniles fed toasted lima bean meal as replacement for Soyabean

Parameters	Treatments						
	1	**2**	**3**	4	**5**	6	**SEM**
Weight Gain (g)	1.75^{ab}	1.79^{ab}	2.20^{a}	2.28^{a}	1.79^{ab}	1.58^{b}	0.25
Feed conversion ratio	2.77^{a}	2.75^{a}	2.52^{a}	2.20^{a}	3.27^{a}	3.06^{a}	0.88
Feed Intake	3.52^{a}	3.51^{a}	3.48^{a}	3.60^{a}	3.82^{a}	4.15^{a}	0.423
Relative Weight Gain	8.29^{b}	9.33^{b}	14.71^{ab}	28.85^{a}	4.80^{b}	7.38^{b}	7.90
Specific Growth rate	8.22^{b}	9.40^{ab}	10.68^{ab}	14.41^{a}	7.72^{b}	6.84^{b}	2.67
Survival (%)	75.00	100	100	100	90	100	

Discussion

From the result of this study, there was an increase in weight gain among the treatments from 0 to 60% replacement level. These results are similar with those reported by [8, 9, 10] who found that the partial substitution of plant sources in fish diets improved growth performance of common carp (*Cyprinus carpio*). Also, the improved growth performance could be likened to the palatability of the diets which could be due to improved processing and the aroma. The increase in weight gain from control diet to treatment 4 (60%LBM) indicated that the fishes were able to convert feed protein to extra muscles. Aliu and Odeh, [11] reported improved growth rate and weight gain at 100% replacement level for *Heterobranchus bidorsalis.* Weight gain and specific growth rate are usually considered as the most important measurement of productivity of diets [12]. At all levels of substitution, the specific growth rate value was different in all the treatments.

The highest specific growth rate was recorded in treatment 4 (14.41) that was fed with diet containing 60% Lima beans meal. This is similar to the observation of [13] in methionine supplemented toasted lima beans fed to *Oreochromis niloticus* in which growth rate was found to be highest for diet containing 50% inclusion level of Lima bean meal. Treatment 6 with 100% replacement level recorded the least weight gain value, this contradicts the observation of [13] in their related study. Also the report of [11] on *Heterobranchus bidorsalis* showed that weight gain was highest with fish in treatment V (100% LBM) and lowest in treatment II (25% LBM). This result also does not agree with the report of [14], who reported that 40% inclusion levels produced highest mean weight gain. 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 6 could have been as a result of the imbalance in the plant protein source. 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 also been shown to improve dietary utilization in legumes [16].

Feed utilization expressed as FCR is known to be affected by body weight, ration and size and temperature [17]. Lower feed

conversion ratio indicates higher protein conversion efficiency thereby resulting in better growth [18]. According to [19] the lower the FCR, the better the feed utilization by the fish. The study showed no significant difference ($P>0.05$) among the six treatments meaning that feed was converted at the same level of intensification within each treatment. However, Treatment 4 had the best feed conversion ratio followed by treatment 3. This study does not correlate with the report of [17], who reported that increasing level of soybean in fish diet resulted in a decrease in FCR. This also is not in agreement with the report of [16] who recorded the best (1.00±0.00) feed conversion ratio for *C. gariepinus* when replacing soybean with Baobab seed at 10%. From this study, feed conversion ratio increased with increasing rate of lima bean meal substitution up to 60% (Treatment 4) after which the FCR decreased with increase in substitution for treatments 5 and treatment 6.

Conclusion

The toasted Lima bean meal diets fed to *Hetero Clarias* had significant effects on their growth and nutrient utilization. The result obtained from this study showed that among the diets with toasted Lima bean meal, diet 4(treatment 4) with 60% inclusion rate had the best performance and feed utilization value as it had the highest value of weight gain. The study has demonstrated that toasted Lima beans can partially replace Soya bean meal and thus reduce the cost of fish feed.

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