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Growth Response of *Heterobranchus longifilis* juveniles Fed *Leucena leucocephala* Leaf Meal Supplemented diets as Replacement for Soyabean

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Abstract
It is important to explore alternative protein-rich resources to supplement traditional feed sources so as to lower feeding costs. *Leucaena* leaf meal (LLM) stands out as a viable option, sourced from the plant *Leucaena leucocephala. L. leucocephala* leaves are available year-round and can be gathered at minimal expense. This study conducted a 56-day feeding trial to assess the growth response of *Heterobranchus longifilis* juveniles (22.33-28.6g) fed varying levels of *Leucaena* leaf meal to replace soyabean. The *Leucaena* leaves were prepared by soaking in water for three days, followed by sun-drying. Experimental diets were formulated using a completely randomized design with five treatments: 0% (SWC), 10% (SW10), 20% (SW20), 30% (SW30), and 40% (SW40) *Leucaena* leaf meal. Results showed that fishes fed with 30% *Leucaena* leaf meal (SW30) exhibited the highest mean weight gain (MWG) of 25.47g±6.08, while those on the control diet (SWC) displayed the lowest MWG of 12.47 ± 2.77. Significant differences (P≤0.05) were observed among treatments for MWG, protein efficiency ratio (PER) and specific growth rate (SGR). Furthermore, carcass analysis revealed significant variations, with protein content being notably higher in fish fed with diet SW30 (46.35±0.99%) compared to those on diets SWC and SW10 (43.89±0.50%). This research underscores the potential of *Leucaena* leaf meal to partially replace soybean meal at a 30% inclusion level, in the diet of *H. longifilis* juveniles.
Keywords: Heterobranchus longifilis, soyabean, Leucena leaf meal, and growth response

Introduction

Aquaculture remains the most effective method for enhancing fish production (WorldFish, 2018). Nutrition, in aquaculture stands out as a paramount factor because feeding expenses accounts for 60–70% of the overall production costs (Aliu *et al*., 2018). Fish meal, soybean meal/cake, and groundnut cake serve as traditional protein sources in fish diets. Numerous studies have indicated that plant-based protein sources possess the ability to supply fish with the necessary protein for optimal growth (Enyidi and Mbenka, 2014). Soybean meal and cake are commonly used as plant protein ingredients in fish feeds owing to their high protein content and digestibility (Eyo, 2003). In spite of their possibility of being replaced or supplement fish meal, the use of soybean meal for human consumption drives up their costs, rendering their use in fish feeds unsustainable (Madu *et al.,* 2003). Consequently, it is imperative to search for other underutilized proteinous plant feed ingredients suitable for fish feeds. Legumes are among these underutilized proteinous plant feed ingredients, offering cost-effective protein feed ingredients that have the potential to reduce the expense of fish diets. Various researchers have explored the substitution of conventional plant protein feed ingredients such as soybeans and groundnut cake with sesame seed, lablab bean, toasted leucaena seed meal, and moringa (Aliu and Osaro, 2018; Jibrin *et al.,* 2018; Ibiyo *et al*., 2020; Fakolade *et al.,* 2021; Jimoh *et al*., 2022).

Leucaena leucocephala is one of the underutilized non-conventional plant protein sources that have the potential to supplement or replace soyabeans in fish feed. *L. leucocephala* is a legume belonging to the *Fabaceae* family, sub-family *Mimosoidae*.). *L leucocephala* is an evergreen multipurpose legume, which is abundant in Nigeria and the leaves can be harvested at minimal cost (Heuze and Tranc, 2012). *Leucaena* leaves have been used to feed other animals, such as laying hen and rabbits (Atawodi *et al.,* 2008; Adedeji *et al.,* 2013). Amisah *et al.* (2009) and Tiamiyu, *et al.* (2015) included *Leucaena* leaves in *Clarias gariepinius* fingerlings and juvenile's diet. *Leucaena* leaf has been reported to have crude protein values ranging from 15.2–34.3% dry matter (Heuze and Tranc, 2012). *Leucaena* leaves contain appreciable quantities of minerals (calcium, potassium and magnesium). It is also rich in essential amino acids, carotene, vitamin A and B (Monoj and Bandyopadhyay, 2007).

Heterobranchus species is an important aquaculture candidate in Nigeria (Ayinla, 2007). This species has great potential to increase fish production and is readily acceptable to fish farmers. *Heterobranchus longifilis* grows to a large size and commands high market value due to its taste and flavor. *H. longifilis* is an omnivore and has the ability to use supplementary feed effectively. The Nigerian farmer is yet to meet the demand of the populace for *H. longifilis* as a result of high cost of feeds, it is imperative to increase the production of this species using affordable feeds. Hence the present research aims to assess the growth of *H. longifilis* juveniles fed different inclusions of *Leucaena* leaf meal to substitute soyabeans.

Materials and Methods

The experiment was carried out between the months of August and September, 2022, at the Fisheries section of the National Open University of Nigeria, Kaduna. This place is situated in Igabi local government area, and located within latitude 9°34'N and longitude 8°17'E. It falls within the Guinea Savannah ecological zone.

Collection and Processing of Leucaena leucocephala Leaves

The leaves of the *Leucaena* plant were harvested from the College of Agriculture and Animal Science forage section, Mando Road, Kaduna, Nigeria by hand. The leaflets were removed from the long leaf stalk, after which 500g of the leaves were treated by soaking in ten litres of water for 72 hours at room temperature (30° C). The water from the leaves was drained, and then the leaves was allowed to dry homogenously in the sun, as described by Amisah *et al*. 2009. The dried leaves were ground into fine powder using a hammer mill, then stored in polythene bags pending proximate analysis. The chemical analysis was done in triplicates as described by AOAC (1990).

Formulation of Experimental Diets

Imported Danish Fishmeal (72% crude-protein), vitamin premix, groundnut cake maize, soyabean cake and bonemeal were purchased from a reputable feed miller in Kaduna**.** Each feed ingredient was weighed according to the formulation (Pearson's square method) and grounded into fine powder to improve pellet quality using a hammer mill. The different feed ingredients were mixed and pelleted into 3mm with a locally fabricated industrial pelleting machine according to methods described by Gabriel *et al.* (2007), then the pellets were sundried and packed into polythene bags before analysis. Five different test diets were formulated, each containing varying amounts of *Leucaena leaf meal*: 0%, 10%, 20%, 30%, and 40%. The control diet, which had no *Leucaena leaf meal*, was labelled SWC, while the diets containing 10%, 20%, 30%, and 40% *Leucaena leaf meal* were labeled SW10, SW20, SW30, and SW40, respectively. Table 1 displays the ingredient composition of these experimental diets.

Experimental procedure

Three hundred juveniles of *Heterobranchus longifis* were purchased from Maidalla farms, Mando Kaduna and transported in 50 litres jerrycans. Following their arrival, they were acclimatized for two weeks. During acclimatization the juveniles were fed with a commercial diet. Subsequently, the fishes were deprived of food for 24 hours before commencing the feeding trial. The juveniles were stocked at 10 juveniles per tank in a completely randomized design with five treatments and three replicates per treatment. Daily maintenance involved siphoning excess feed and feaces from the tanks. After the acclimatization period, 150 fish were randomly chosen, weighed in batches and placed into fifteen 60 liters capacity circular plastic tanks. These tanks were filled to 50 liters with water sourced from a borehole and stored in overhead plastic tanks. Water replacement occurred twice weekly. To prevent fish from escaping, the tanks were securely covered with net. Left over feed and feaces were flushed out daily while any dead fish found was removed and recorded. Fishes were fed 2% body weight twice daily for 56 days. The ration was recalculated after every two weeks according to the weight increase recorded when the fishes were sampled. Water quality parameters were taken every two weeks before sampling. Water temperature, oxygen levels and pH were assessed using a portable dissolved oxygen meter (TPB-607) and a pH meter (model pH-009).

Growth Parameters

The following parameters were calculated as described by Castell and Tiews (1980) to determine the growth response of *H. longifis*.

Mean Weight Gain (MWG) = Mean final weight – Mean initial weight

Feed Conversion Ratio (FCR) = Total Feed consumed/ eight gain by fish

Survival rate = Total number of fish harvested (100/initial number of fish stocked).

Specific Growth Rate (SGR) =

$$\ln W1 - \ln W2 \times {}^{100}/_{T2 - T1}$$

Where W2 = Final weight, W1 = Initial weight

T2-T1 = Time interval between W2 and W1 in days

ln = Natural log

Chemical Analysis

The composition of both the experimental feed and the fish was analyzed using the method outlined in AOAC (1990).

Statistical Analysis

The data obtained underwent one-way analysis of variance (ANOVA) using SPSS version 15.0 to determine any significant differences. Additionally, Duncan's multiple range test (DMRT) was employed to identify the means that were significantly different. A significance level of $P \leq 0.05$ was set for treatments.

Results

Table 2 presents the proximate composition of the experimental feeds and *Leucaena leaf meal. Leucaena* *leaf meal* exhibited a crude protein value of 22.08%, while the experimental feeds ranged from 28.12±0.50% to 31.57±2.95% crude protein. The mean weight gain (MWG) was highest in treatment SW30 which was 27.45g±6.08 and lowest in SWC which was 11.62g±2.77. A variation in MWG was observed between the control (SWC) and the treatments having varying levels of *Leucaena* leaf meal, demonstrating statistical significance (P≤0.05). Similarly, the specific growth rate (SGR), the protein efficiency ratio (PER) and mean feed intake (MFI) followed the same pattern; these parameters were higher in the *Leucaena* supplemented diets than the control and also were significantly different (P≤0.05). Although the feed conversion ratio (FCR) was relatively high in fishes fed control group and lowest in fish fed the SW30 diet, there was no significant difference (P>0.05) among all treatments. There was no significant difference (P>0.05) for survival rate (SR) in all the treatments. The mean water quality parameters are summarized in Table 4.

The carcass composition of H. longfilis varied across the different experimental diets. Protein content increased from the initial 41.90±0.49% to 43.89±0.50%, 44.87±0.49%, 45.86±0.50%, and 46.35±0.99% for diets SWC, SW 10, SW 20, SW 40, and SW 30, respectively. However, the protein content was higher and exhibited significant difference (P≤0.05) in fishes that were fed diet SW30 (46.35±0.99%) compared to those fed diets SWC and SW 10 (43.89±0.50%). The fat (Ether extract) content showed significant difference (P≤0.05) between the control group and fish fed SW 10, SW 30, and SW 40. Fishes in SW 40 had the highest fat content whereas the lowest was in fishes fed SW 30. Furthermore, the ash content was higher in fishes that were provided with the control diet SWC in comparison to those that consumed diets containing *Leucaena* leaf meal and demonstrated a significant difference (P≤0.05).

Discussion

The protein content of *Leucaena* leaf meal in this work compared with findings from previous research (Agupugo *et al*., 2022). While Amisah *et al.* (2009) found a higher value when *Clarias gariepinus* fingerlings were fed diets containing *Leucaena* leaves. These differences could be due to variations in processing methods applied to the leaves, as indicated by Agbo *et al*. (2017), who noted different crude protein levels for sundried and soaked *Leucaena* leaf meal. The protein content of the experimental diets fell within the range documented for juvenile catfish in prior research (Amisah *et al*., 2009). The increase in growth from initial weight suggests that *H. longifilis* could tolerate up to a 40% inclusion level of *Leucaena* leaf meal and utilize it for growth. This observation could be due to the combination of different protein sources in the experimental diets, as observed by Sogbesan and Ugwumba (2008) when using a blend of fishmeal, groundnut cake, and termite meal in *H. longifilis* diets. The overall weight gain in all treatments implies effective conversion of feed protein into muscle, which is consistent with findings by Eyo and Olatunde (2001). Amisah *et al*. (2009) noted an increase in mean weight gain of *Clarias gariepinus* as *Leucaena* leaf meal inclusion increased, a trend also observed by Tiamiyu *et al*. (2015) for *Clarias gariepinus* fingerlings. However, Fakolade *et al*. (2021) observed a decrease in mean weight gain with increased levels of *Leucaena* seed meal in *Clarias gariepinus* fingerling diets. Similarly, Aliu and Osaro (2018) observed retarded growth in *Heteroclarias* fingerlings fed *Lablab* Bean Meal diets due to residual antinutrients. The increased weight gain in fishes fed *Leucaena* leaf meal-based diets in this study aligns with Bello's (2013) assertion that low leaf meal protein substitution levels (less than 50%) in fish diets supports growth. This observation is supported by Ibiyo *et al*. (2020), who reported that *M. olifera* leaves could partially replace soybean meal in *H. longifilis* diets at 5–10% inclusion level.

Specific growth rate (SGR) and Protein efficiency ratio (PER) followed the same increasing trend observed in mean weight gain (MWG). The variation and increase in PER of *Leucaena* leaf meal-based diets suggest efficient conversion of consumed protein into weight gain, which is in line with the findings of Adesina *et al.* (2013) and supported by Afe *et al*. (2019), who reported higher PER values in *Heterobranchus bidorsalis* fed *Eucalyptus globulus* leaf meal-supplemented diets. In this study, the PER value was close to the values reported by Otchoumou (2019) for *Heterobranchus longifilis* fed different levels of *Nymphaea lotus* meal. SGR values were similar to those observed by Adesina *et al*. (2013), and the higher SGR in fishes fed *Leucaena* leaf meal-based diets over the control aligns with the report of Tiamiyu *et al.* (2015).

The more efficient a feed is utilized by the fish, the lower the feed conversion ratio (FCR) (Adikwu, 2003). Findings from the study revealed that the FCR values were lower than those reported for *H. longifilis* fed *Nymphaea lotus* meal diets and when *Leucaena* leaf meal was *included in Clarias gariepinus* fingerling diets (Tiamiyu *et al*., 2015; Otchoumou *et al*., 2019). Variations in FCR across studies could be due to different rearing conditions, fish species, health, environmental factors, feed preparation, feed intake and inclusion of other plant-based ingredients in the diet, as noted by Robinson and Menghe (2015). The FCR was close to the suggested level for cultured catfish by Robinson and Menghe (2015). The high survival rate could be attributed to the favourable water quality parameters which were within recommended range for catfish.

Protein requirement is crucial in nutritional studies due to its importance for growth and development, as well as being the most expensive in diet formulation. All diets in this study increased fish carcass protein values when compared to initial values, indicating diet utilization, as observed by Aliu *et al.* (2018) when *Heteroclarias* was fed *Lablab* leaf meal-based diets**.** Alegbeleye *et al.* (2001) also observed that different rates of Bambara groundnut utilization resulted in varying levels of protein and lipid in *Heteroclarias* carcass. Although the crude protein of fish carcass in this study was lower than values obtained by Afe *et al*. (2019) for *Heterobranchus bidorsalis* fingerlings fed graded levels of *Ocimum gratissimum* Leaf Powder, it was close to values reported by Adamu et al. (2021) for hybrid catfish fed diets containing cockroach meal. Variation in carcass lipid between control and *Leucaena* leaf meal-based diets could be attributed to complexes formed with antinutrients (Osuigwe and Obiekezie, 2007). The partial replacement of high-cost soybean meal in this study confirms earlier findings that soybean meal can be substituted with other non-conventional ingredients in fish (*C. gariepinus, Heteroclarias* and *Heterobranchus*) diets without negatively impacting growth performance (Bamidele *et al*., 2015; Aliu *et al*., 2018).

Conclusion

The findings from this study revealed that Leucaena leaf meal can partially substitute soyabean in the diet of juvenile H. longifilis without adversely affecting their growth. However, for optimum production, a 30% inclusion level is suggested in the diet of H. longifilis juveniles.

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Table 1: The Ingredient Composition of Experimental Diets

Ingredients (g/100g)	SWC	SW10	SW20	SW30	SW40
Fishmeal	30	30	30	30	30
Maize	20	20	20	20	20
Groundnut cake	10	10	10	10	10
Soyabean cake	38	34.2	30.4	26.6	22.8
Leucaena leaf meal	-	3.8	7.6	11.4	15.2
Bonemeal	1	1	1	1	1
Methionine	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 2: Proximate composition of experimental feeds and *Leucaena* leaf meal

		Treatment				
Parameter (%)	SWC	SW 10	SW 20	SW 30	SW 40	Leucaena leaf meal
Dry matter	88.82±4.56	93.77±0.10	93.11±0.82	93.16±0.10	93.28±0.05	8.15±0.22
Crude protein	28.22±0.45	28.12±0.50	28.25±0.50	28.69±3.90	31.57±2.95	22.08±1.30
Crude fiber	3.86±0.66	3.56±0.47	3.56±0.45	4.32±0.48	5.13±0.43	5.37±0.25
Ether extract	3.13±0.69	3.03±0.07	2.93±0.08	3.22±0.44	3.69±0.21	21.5±2.73
Ash	9.50±1.50	11.47±1.14	9.10±2.50	10.33±0.33	9.29±1.96	5.56±0.81
NFE	54.80±2.49	53.70±0.96	56.15±2.50	46.88±2.66	46.18±1.96	38.33±4.66

Table 3: Growth parameters of *H. longfilis* fed graded levels of *Leucaena* leafmeal

		Treatment			
Parameter	SWC	SW 10	SW 20	SW 30	SW 40
IWT(g)	22.33±0.00^{a}	25.93±3.35^{a}	23.00±0.00^{a}	26.60±3.46^{a}	26.97±2.22^{a}
FWT(g)	33.95±2.35^{b}	43.53±3.07^{ab}	45.63±2.30^{ab}	54.07±6.08^{a}	50.43±11.74^{a}
MWG(g)	11.62±2.35^{b}	17.60±1.31^{ab}	22.63±2.30^{ab}	27.45±6.08^{a}	23.46±12.43^{ab}
SGR (%/day)	0.34±0.06^{b}	0.47±0.07^{ab}	0.53±0.04^{ab}	0.55±0.03^{a}	0.44±0.11^{ab}
PER	0.95±0.17^{b}	1.34±0.14^{ab}	1.57±0.11^{ab}	1.72±0.26^{a}	1.37±0.69^{ab}
FCR	2.37±0.48^{a}	1.66±0.14^{a}	1.46±0.11^{a}	1.38±0.23^{a}	2.25±1.62^{a}
MFI	28.69±2.16^{c}	32.22±2.62^{bc}	33.06±1.43^{bc}	34.37±3.31^{ab}	37.49±1.96^{a}
SR (%)	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	90.00±10.00^{a}	93.00±5.77^{a}

Means having the same superscript within a row are not statistically significant (P>0.05).

Table 4: The mean water quality parameters

			Treatments		
Parameter	SWC	SW 10	SW 20	SW 30	SW 40
DO (mg/l)	1.3±1.1	0.5±0.1	0.56±1.5	0.56±0.11	0.56±0.11
Ph	6.68±0.72	7.33±0.31	7.35±0.35	6.95±0.07	6.92±0.42
Temperature	26.40±1.85	24.30±0.00	24.30±0.00	24.66±0.63	24.66±0.63

Table 5: Carcass composition of *H. longfilis* fed graded levels of *Leucaena* leafmeal

			Treatment			
Parameter	Initial	SWC	SW 10	SW 20	SW 30	SW 40
Dry matter	32.94±0.44^{a}	23.27±1.19^{c}	25.76±1.48^{bc}	24.18±2.21^{c}	25.56±1.25^{bc}	29.07±3.00^{b}
Crude protein	41.90±0.49^{d}	43.89±0.50^{c}	43.89±0.50^{c}	44.87±0.49^{bc}	46.35±0.99^{a}	45.86±0.50^{ab}
Crude fiber	ND	ND	ND	ND	ND	ND
Ether extract	6.19±0.01^{b}	6.50±0.52^{ab}	6.85±0.27^{a}	6.67±0.08^{ab}	5.50±0.30^{c}	6.89±0.18^{a}
Ash	13.95±0.06^{bc}	15.71±0.41^{a}	13.73±0.07^{c}	14.08±0.60^{bc}	14.81±1.21^{ab}	13.35±0.46^{c}
NFE	37.95±0.55^{a}	33.91±0.39^{c}	35.54±0.84^{b}	34.38±0.63^{c}	33.34±0.52^{c}	33.94±0.22^{c}

Means having the same superscript within a row are not statistically significant (P>0.05).