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Assessment of Growth and Nutrient utilization in *Clarias gariepinus* x *Heterobranchus bidorsalis* hybrid (Heteroclarias) fingerlings- fed *Leuceana leucocephala* (Lam. de Wit) seed Meal

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Abstract

There is a high demand for the plant protein source, soybean, for direct human consumption and industrial uses. A feeding trial was carried out to assess the growth and nutrient utilization of Clarias gariepinus x Heterobranchus bidorsalis hybrid (heteroclarias) fingerlings fed toasted Leuceana leucoceplala (Leuceana) seed meal in replacement for soybean meal. One hundred and five Heteroclarias fingerlings of average weight 3 ± 0.14g were fed five isonitrogenous (40% crude protein) diets in which toasted Leucaena seed meal replaced soybean meal at 0% ($g.100g^{-1}$), 20%, 40%, 60%, and 80% respectively as diets I – V for 8 weeks. Proximate analysis of the diets was done before the experiment. Cleaning of experimental units was carried out by siphoning solid waste in each unit and water reduced by half and replaced before feeding. Fishes were fed in triplicates twice daily to satiation. Data obtained were subjected to ANOVA and means were deemed significant at 5% probability level. There was no significant difference ($p>0.05$) in mean weight gain (MWG), daily weight gain (DWG), and specific growth rate (SGR), as well as nutrient utilization indices – feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) between the control diet and 20% inclusion. A further increase in Leuceana inclusion resulted in a progressive decline in growth and nutrient utilization and were significantly different from the control and 20% inclusion. Findings from this study suggest that soybean meal could be substituted with 20% Leuceana seed meal in the diet for heteroclarias fingerlings.

Keywords: Growth decline, Heteroclarias, *Leuceana leucocephala*, Plant protein

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INTRODUCTION

Despite breakthroughs recorded in fish breeding, rearing facilities, accessories design and construction, farm bio-security, and general farm management, achieving the aim of harvesting the maximum weight of fish from a given unit of culture facility in the shortest possible time making the most efficient use of inputs to maximize profit is yet an arduous task. This is because of the scarcity of feeds, the most expensive cost item accounting for about 40-60% of the total expenditure and 60-80% of the operation costs (Hasan and Shipton, 2021). Thus, slowing the pace of aquaculture production. There is a need for low-cost fish feed formulated and compounded from nonconventional local feed resources as indicated by the high and rising cost, dwindling supply, and competition between man, livestock, and industries for conventional feedstuffs.

The increasing demand for human consumption, industrial use, and export potential of, a predominantly used plant protein source in fish feed, soybean, has led to its scarcity and attendant high cost. Leuceana is a multipurpose leguminous tree that flowers all year round and is capable of producing about 3-5 tons of seed per hectare (Cardona *et al*., 2015; Brewbaker and Hutton, 2019). The high protein and β-carotene content is accompanied by an amino acid composition like that of soybean meal and fish meal which is rich in essential amino acids such as isoleucine, leucine, phenylalanine, and histidine (De Angelis *et al*., 2021). Leucaena offers a good potential as a cheaper plant protein source in fish diet with high nutritive value (Agupugo *et al*., 2022). Al-Amin *et al*. (2019) concluded that a pelleted diet containing 10% leucaena leaf meal, as a replacement for soybean meals and copra cake in complete feed, improved the growth performance of New Zealand White rabbit males. Soaked Leucaena leaves at 25% inclusion in the diet of *Labeo rohita* resulted in better feed acceptability, growth, feed conversion, protein utilization and digestibility, and body composition (Hasan *et al*.,1994). Soaked and dried Leucaena leaf meal with up to 20% inclusion replacing fishmeal and soybean meal in diets for African catfish fingerlings resulted in better growth, feed conversion ratio, protein efficiency, and survival rate (Tiamiyu *et al.,* 2015). The seed is high in protein (24-46%) with a rich amino acid profile, low in oil (5-10%) about 73% unsaturated fatty acid, and rich in calcium and phosphorus (Ahmed and Abdelati, 2009). Aliu and Obuseli (2021) recommended 20% inclusion in the diet for *Clarias gariepinus* fingerlings. Ananda *et al.* (2022) reported that fermented leuceana seed meal in feed increased the growth and protein content of gourami's juvenile at 40 % inclusion. However, Fakolade *et al*. (2021) reported a decrease in mean weight gain, feed intake, relative weight gain, Specific growth rate, and feed conversion ratio with increased leuceana seed inclusion in *Clarias gariepinus* diet.

The choice of fish species to culture to realize the goal of producing table-sized fish within the shortest possible time is cardinal. Heteroclarias is an inter-specific hybrid of *Clarias gariepinus* and *Heterobranchus bidorsalis* (Bartley *et al*., 2000). It exhibits superior growth, improved survival, and general hardiness than the pure breeds (Ayanwale *et al.,* 2020). The blending of high survival and fast growth in the hybrid catfish (*Heteroclarias*) offers higher production prospects (Afia and David, 2019). It is very profitable because of its high resistance to diseases and environmental stress (Ayanwale *et al*., 2021). Its potential for contributing to increased food supply and farm income makes heteroclarias an important aquaculture candidate. Thus, the need to explore the productive potential of leuceana seed meal in heteroclarias nutrition. This study investigated growth and nutrient utilization in Heteroclarias fingerlings fed varying levels of toasted *Leuceana leucocephala* seed meal.

MATERIALS AND METHODS

The experiment was carried out at the Teaching and Research farm of the Department of Aquaculture and Fisheries Management, University of Benin for eight weeks.

Preparation of Leucaena and Soyabean Meal

Leucaena seeds were harvested from the *L. leucocephala* shrubs in the University of Benin environment. Matured and dried pods were harvested by hand and shredded to release the seeds. The whole seeds were then sundried for five days at ambient temperature to dry properly. Fresh soybeans were purchased at the New Benin market in Benin City. Leuceana and soybean seeds were then toasted respectively following the methods of Akpotor *et al*. (2023). The toasted seeds were first crushed with a hammer mill to separate the testa which were winnowed off before the kernels were milled to fine powder to obtain the respective seed meals.

Preparation of Experimental Diets

Fishmeal, corn meal, wheat offal, bone meal, vitamin premix, and palm oil were purchased from Jappa Feeds Limited and Osa market in Benin City. Five iso-nitrogenous (40 % crude protein) and isocaloric diets in which leucaena seed meal substituted soybean meal at 0%, 20%, 40%, 60%, and 80% respectively were formulated to give diets I - V. Diet I (0%) was the control. The gross ingredient and analyzed composition of the diets is shown in Table 1. The required quantity of the finely ground ingredients for the diets were weighed according to their calculated weight and mixed thoroughly. The corn meal was gelatinized in hot water and used as a binder. The dough were then pelleted with a 2mm die and dried with the electric feed drier and stored in air-tight containers.

Table 1. Formulations and analyzed composition of experimental diets.

Ingredients ($g.100g^{-1}$)	Diets				
	I (0%)	II (20%)	III (40%)	IV (60%)	V (80%)
Fish meal	35.00	35.00	35.00	35.00	35.00
SBM	35.00	28.00	21.00	14.00	7.00
LSM	0.00	7.00	14.00	21.00	28.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00
Corn meal	10.00	10.00	10.00	10.00	10.00
Wheat offal	4.00	4.00	4.00	4.00	4.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Vitamin/Mineral premix	2.00	2.00	2.00	2.00	2.00
Bone meal	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Analyzed composition (%)					
Dry matter	90.90	90.86	90.96	91.03	90.98
Crude protein	40.31	40.17	40.08	40.15	40.06
Ether extract	6.80	6.76	6.30	6.50	6.10
Fibre	6.45	6.52	7.01	6.74	7.16
Ash	10.76	11.26	11.04	11.60	11.43
NFE	26.58	26.38	26.53	26.10	26.25
Gross energy (k $cal.100g^{-1}$)	425.35	423.65	421.43	420.84	418.90

SBM-soybean meal, LSM-leuceana seed meal, NFE – nitrogen free extract, % - percentage substitution of LSM for SBM.

Experimental design

One hundred and five healthy Heteroclarias fingerlings of average weight 3 ± 0.14g were obtained from the hatchery unit of the Departmental farm and acclimatized for two weeks in the Laboratory, during which they were fed commercial diets. After acclimation, they were weighed in batches of 7 each into experimental plastic tanks measuring (60 cm x 28 cm x 28

cm) and each filled with water up to 2/3 of its volume. The tanks containing the fish were now randomly assigned in threes to each diet and properly labeled, giving five treatments in triplicates. Feeding was by hand to satiation at 9:00 am and 4:00 pm daily. The experimental units were cleaned daily by siphoning to remove solid wastes.

Water quality management

Solid waste in each unit was siphoned and the water reduced by half and replaced daily before fish were fed to provide more dissolved oxygen. Total replacement of water with fresh clean water was done during fish growth data collection. Water quality parameters viz., Temperature, Dissolved oxygen, and pH were monitored and recorded. Mean values are shown in Table 2.

Table 2. Mean values of water quality parameters recorded during the experimental trial

Parameters	Mean ± SD
Temperature (^oC)	25.40 ± 0.01
Dissolved oxygen ($mg.L^{-1}$)	6.10 ± 0.04
pH	7.60 ± 0.02

Assessment of growth performance

Sampling was done once in two weeks to assess growth performance. Data on feed consumed and weight gain were collected from which feed intake (FI), feed conversion ratio (FCR), protein intake (PI), protein efficiency ratio (PER), mean weight gain (MWG), specific growth rate (SGR), daily weight gain (DWG) and survival rate (SR) were evaluated following the formulae of Ekelemu *et al*. (2022) and Sridharan *et al.* (2023).

Proximate analysis of diets

Diet samples were analyzed using standard methods of the Association of Official Analytical Chemists (AOAC, 2019) for moisture, crude protein, lipid, crude fibre, and ash. A difference [100 – (percentage of moisture + crude protein + ether extract + crude fibre + total ash)] was used to determine the level of nitrogen-free extract (NFE).

Statistical analysis

All data obtained were analyzed by one-way ANOVA using statistical software SPSS version 20.0. Differences in treatment means were deemed significant at 5% level of probability and separated using Duncan's multiple range test.

Results

Growth Performance

Presented in Table 3 is the growth performance results of hybrid catfish heteroclarias fingerlings fed *Leuceana* seed meal diet. At the end of the feeding trial, survival ranged from 89.37% in diet V to 91.97% in diet II with no significant variation across the treatments ($P>0.05$).

Table 3: Growth performance of Heteroclarias fingerlings fed *L. leucocephala* seed meal diets.

Diet	Survival Rate (%)	Initial mean weight (g)	Final mean weight (g)	MWG (g)	DWG (g day^{-1})	SGR (% (day^{-1})
I (0%)	91.54±1.72	21.00± 0.27	36.37±0.57^a	15.41±0.63^a	0.27±0.00^a	0.97±0.03^a
II (20%)	91.97±1.53	21.00± 0.27	35.93±0.07^a	14.88±0.12^a	0.26±0.00^a	0.95±0.01^a
III (40%)	90.31±1.17	21.00± 0.27	30.77±0.61^b	9.51±0.43^b	0.17±0.01^b	0.68±0.03^b
IV (60%)	89.62±1.94	21.00± 0.27	27.23±0.35^c	6.47±0.47^c	0.11±0.00^c	0.46±0.02^c
V (80%)	89.37±0.65	21.00± 0.27	26.14±.12^d	5.04±0.06^d	0.09±0.00^d	0.39±0.01^d

Data are presented as Mean ± SD. MWG – weight gain, DWG – daily weight gain, SGR – specific growth rate.

The MWG, DWG, and SGR were highest in diet I and least in diet V, and were significantly (P<0:05) affected by the leuceana seed meal inclusion levels in the diets giving the progressive decline with an increase in leuceana seed meal inclusion. At 20% inclusion, leuceana seed meal gave similar results (P>0:05) as the control diet. However, increased inclusion resulted in a decline in MWG, DWG, and SGR.

Feed utilization

Table 4: Nutrient utilization of Heteroclarias fingerlings fed *Leuceana leucocephala* seed meal diets.

Treatment	FI (g)	FCR	PER	PI
0%	18.39±0.68^{a}	1.19±0.01^{a}	2.09±0.01^{a}	7.35±0.27^{a}
20%	17.80±0.65^{ab}	1.19±0.03^{a}	2.08±0.06^{a}	7.12±0.26^{ab}
40%	17.81±2.08^{ab}	1.86±0.17^{b}	1.34±0.12^{b}	7.12±0.83^{ab}
60%	15.06±1.38^{c}	2.34±0.37^{c}	1.07±0.18^{c}	6.02±0.55^{c}
80%	15.67±1.39^{bc}	3.10±0.27^{d}	0.80±0.07^{d}	6.27±0.56^{bc}

FI – feed intake, FCR – feed conversion ratio, PER – protein efficiency ratio, PI – protein intake

Feed utilization results of heteroclarias fingerlings fed *Leuceana leucocephala* seed meal diet showed that FI and PER were highest in diet I and least in diet V. FCR increased progressively from 1.19 ± 0.01 in diet I to 3.10 ± 0.27 in diet V. Leuceana inclusion level significantly affected (P<0.05) feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) as there was decline in the values when leuceana was increased beyond 20% in the diet. However, at 40% inclusion FI was not significantly different (p>0.05) from the control.

DISCUSSION

The superior values obtained for feed intake, feed conversion and protein efficiency in diets I and II relative to other diets is an indication of better feed acceptance, digestibility and utilization. The decline in feed intake beyond 20% inclusion could be attributed to mimosine content of the leuceana substituted diets which may have risen as the substitution level was increased. Mimosine limits the intake of a high amount of *Leucaena leucocephala* seeds (Wardatun *et al.*, 2020).

Incomplete deactivation and detoxification of anti-nutrients in feedstuff by processing methods results in poor digestibility and utilization of their nutrients contents. The low digestibility and utilization of toasted leuceana seed could have resulted from the residual mimosine content which was not sufficiently detoxified by toasting as also reported by Sotolu and Faturoti, (2008). Conversely, Ananda *et al.* (2022) reported that fermented leuceana seed flour increased growth and protein content of gouramy juveniles when fed at 40% inclusion. The observed better growth performance in diets I and II compared to the higher inclusion levels resulted from the digestibility and utilization of the feed nutrients in building muscle tissues which manifested in the comparative weight gain recorded. This is similar to (Sotolu and Faturoti, 2008) who reported that variation in the mean weight gain resulted from the extent of the digestibility of the feed when they assessed the digestibility and nutritional values of differently processed leucaena seed meals in *C. gariepinus* diet. The decline in weight gain in heteroclarias fingerlings fed leuceana seed meal beyond 20 % inclusion as recorded in this study was not different from that reported by Aliu and Obuseli, (2021) when they studied nutrient utilization and growth parameters in *C. gariepinus* fed leuceana seed meal. Depressed feed intake and weight gain were also observed when Ahmed and Abdelati, (2009) included leucaena seeds in the diet of broilers. Also, earlier findings by Sethi and Kulkarni (1995), Whitesell and Parrotta (2008), and Nursiwi *et al*. (2018) held that though rich in protein, leuceana seed consists of high amounts of mimosine, an amino acid that causes growth

retardation by interfering in the metabolism of some amino acids thus inhibiting protein synthesis.

In this study, heteroclarias fingerlings fed leuceana seed meal at 20 % inclusion in the diet in replacement for soybean meal performed equally as the control. It thus indicates that the optimum inclusion level of leuceana seed meal in replacement for soybean meal in heteroclarias diet is 20%. This finding is similar to Agupugo *et al*., (2022), and Thi and Thi (2023) who found that leuceana leaf powder was efficacious and more cost-effective to replace 20% fish meal in the diet for *C. gariepinus* and white-leg shrimp respectively. These were not also different from Sotolu (2010) and when he included leuceana seed meal in *C. gariepinus* fingerlings diet. Babalola and Fakunmoju, (2020) and Haetami *et al.* (2023) equally reported significant weight gain with leauceana leaf meal in Tilapia diet at 10% inclusion. The inferior growth and nutrient utilization observed in this trial is in line with Sotolu and Faturoti, (2011) who recorded same when leuceana seed meal was included in the diet for *C. gariepinus* juveniles. Also, Verma *et al*., (2018) reported that it improved immune response in *C. gariepinus* but resulted in low specific growth rate.

CONCLUSION

Heteroclarias fingerlings fed diets replacing soybean meal with leuceana seed meal at 0%, 20%, 40%, 60%, and 80% respectively showed high survival at all inclusion levels. However, there was a decline in growth and feed nutrient utilization indices beyond 20% inclusion. Therefore leuceana seed meal is a potential plant protein source to replace soybean meal in the diet of heteroclarias up to 20%.

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