Growth Performance of *Clarias gariepinus* (Burchell, 1822) Fed Varying Inclusions of *Leucaena leucocephala* Seed Meal

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Keywords: Plant protein- *Leucaena* seed meal- Soya bean meal- Catfish feeding- Aquaculture- Nigeria

Summary

This study examined the utilization of Leucaena leucocephala *(Lam de Wit) seed meal (LSM) for sustainable fish production. Six isonitrogenous; 40% crude protein diets were formulated where LSM replaced Soya bean meal (SBM) at 0%, 20%, 40%, 60%, 80% and 100% inclusions. Catfish fingerlings (5.21 ± 0.14 g) stocked at 25 fish/70 liters tanks were fed diets in triplicates three times daily for 112 days. Data from the completely randomized experiment were subjected to ANOVA and correlation analysis was separated at 5% probability level. Mean weight gain (MWG) and Specific growth rate (SGR) of fish fed 20% LSM were statistically different (p< 0.05) from those fish fed LSM at higher inclusion rates. Fish MWG, SGR, PER and FCR significantly (p< 0.05) correlated negatively with LSM inclusion rates r= -0.62,-0.57, -0.78 and -0.64 respectively. Fish carcass proteins of fish were statistically the same for 0%, 20% and 40% LSM fed fish. In the present study processed leucaena seed meal can be considered as a good alternative raw material in substitution to soya bean meal for Clarias gariepinus fingerlings' diets at 20% inclusion level.*

Résumé

Performance de croissance et hématologie de *Clarias gariepinus* (Burchell, 1822) nourri de graines de *Leucaena leucocephala*

Cette étude a examiné l'utilisation de repas à base de graines de Leucaena leucocephala *(LSM) pour une production durable de poisson. Six repas isoazotés à 40% de protéines brutes ont été formulés où LSM a remplacé l'aliment Soja (SBM) au taux de 0%, 20%, 40%, 60%, 80% et 100%. Des alevins de poisson-chat (5.21 ± 0.14 g) stockés à 25 poissons par aquariums de 70 litres ont été alimentés en triplicats, trois fois par jour pendant 112 jours. Les déchets solides ont été siphonnés tous les jours avant l'alimentation avec renouvellement total d'eau lors des prises de données sur la croissance du poisson. Le gain de poids moyen (MWG) et le taux de croissance spécifique (SGR) du poisson nourri à 20% LSM était statistiquement différent (p< 0,05) de ceux à taux d'inclusion plus élevés. Les valeurs MWG, SGR, PER et FCR sont négativement corrélés taux d'inclusion de LSM, r= -0.62,-0.57,-0.78 et -0.64 respectivement. Les taux de protéines de la carcasse de poisson, ont été statistiquement les mêmes pour 0%, 20% et 40% LSM. Dans cette étude, le régime à base de graines de leucaena traitée peut être considéré comme une bonne alternative de matière première en substitution de la graine de soja pour l'alimentation d'alevins de* Clarias gariepinus *à un niveau d'inclusion de 20%.*

Introduction

Lack of readily available nutritive fish feed ingredients have continued to be a major constraint to the survival of aquaculture in the competitive global food production system (8, 15). Consequently, fish nutrition experts world over have considered the recruitment of alternative protein feed ingredients necessary for inclusion in fish diet. Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (10, 14). However, in the compounding of fish ration with plant protein sources, cautions need to be exercised as to their inclusion levels in fish diets as well as ensuring their proper processing for effective utilization (9, 17). The need for such recommendations have been due to the presence of certain limiting factors in those ingredients such as high crude fiber content (14), antinutritional factors such as in *Vigna subtarreana* (1). Studies have shown that, excessive consumption of plant protein sources by fish could cause slower growth rates and poor performance which may result in mortalities if condition persists (4, 9). *Leucaena leucocephala* (Lam de Wit) demonstrated good potential to serve as a useful plant protein source in fish ration and in the livestock industry generally (6, 12, 24). However, it has been established that leucaena contain mimosine-a

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non-protein amino acid capable of inhibiting protein biosynthesis in animal causing growth retardation if consumed intensively (6, 24). Cruz and Laudencia (5) found out that 33 to 100% leucaena leaf meal as integral part of supplemental feed enhanced the growth of *Oreochromis niloticus* (Linn) fingerlings in Lake Laguna while D'Mello and Acamovic (6) stressed its potential as a good feed ingredient in the culture of mollies and topminnows (*Poecilia* spp.) and freshwater prawn (*Macrobrachium rosenbergii*, Linn). A preliminary study by Sotolu and Faturoti (13) revealed that catfish was able to digest leucaena seed meal (LSM) processed by soaking in water than those LSMs processed by other methods. Leucaena seed meal was therefore used to replace the much expensive and scarce soybean meal (SBM) in catfish diets in this study. It involved the evaluation of the effects of leucaena seed meal on growth performance of *Clarias gariepinus*, protein utilization and carcass composition.

Material and methods

Preparation of leucaena seed meal and experimental set-up

Whole pure leucaena seeds were soaked in cold water at 1 kg/5 litres of water for 72 hours as described by Padmavathy and Shobha (16). Seeds were later thoroughly washed in fresh cold water after removal thorough sieve. Seeds were spread in thin layer on a slab for quick and homogenous solar drying for two days. Samples of soaked in cold water leucaena seeds (Processed leucaena Seed Meal, processed LSM) were chemically analyzed according to A.O.A.C. (3). Parameters of importance include crude protein, crude fibre, fat, ash, moisture and calorific value. Six isonitrogenous diets were prepared (40% CP) where the processed leucaena seed meal (LSM) replaced soyabean meal (SBM) at 0%, 20%, 40%, 60%, 80% and 100%. All ingredients used in the formulation were ensured constant except SBM and P-LSM that were made to substitute for one another on percentage basis shown. Slight variations however occurred in the crude protein content of the formulated diets on chemically analysis and this may be due to differences in their compositions. Values of their crude protein ranged between 39.86% and 40.29%. Chemical analysis of experimental feeds and fish were done before feeding trials using the method of A.O.A.C. (3) while that of the experimental fish alone was repeated at the end of the experiment. Water quality parameters (pH, dissolved oxygen, ammonia) were kept favourable for fish by constant renewal of water in fish tanks at a flow rate of 2.5 l/min till the end of the experiment. Catfish fingerlings (5.21 ± 0.14 g) were stocked in a circular tank of 70 l at 25 fish/tank in triplicates. Feeding commenced three days after acclimation of fish sourced from the University of Ibadan fish hatchery. Fish were fed to satiation between the hours of 09:00 h-09:30 h, 14:00 h-14:30 h and 19:00 h-19:30 h daily for 112 days. Solid wastes were siphoned out of tanks everyday before feeding and total cleaning of fish tanks and replacement with clean fresh water was done every fortnight.

Determination of feed utilization and data analysis

Data were collected fortnightly on fish growth performance and nutrient utilization by determining mean weight gain [(MWG) g], Gross energy was calculated according to Jobling (11) with multiplier factors of carbohydrate, 4.1 kcal/g, protein, 5.4 kcal/g and lipids, 9.5 kcal/g. SGR= 100 x (Loge final weight(g) – Loge initial weight(g)/culture period (days), Feed Conversion ratio-FCR= Total feed fed(g)/total wet weight gain(g), Protein Efficiency ratio-PER= wet weight gain (g)/amount of protein fed, Nm= (0.549) (a+b) h/2; where a= initial mean weight (g) of fish, b= final mean weight (g) of fish and h= experimental period in days, NPU= Nitrogen content of fish after experiment-Nitrogen content of fish before experiment + Nitrogen metabolism/Nitrogen of experimental diet and, Survival rate (SR) = 100 (Number of fish stocked-Mortality) / Number of fish stocked. Data collected were subjected to ANOVA and correlation analysis using the SPSS package version 10 and significant mean differences were separated at 0.05 probability levels according to Steel *et al.* (20).

Results

Proximate composition of processed and unprocessed leucaena seeds were 36.01% and 22.75 crude proteins respectively while the lipid contents are 5.18% and 6.12% respectively. Ash and crude fiber contents for processed LSM were 3.74% and 7.11% respectively while they were 5.98% and 11.38% for unprocessed LSM respectively. Gross energy for processed LSM (2899.76 kcal/kg) was higher than that of unprocessed LSM (2833.50 kcal/kg). Gross and proximate compositions of the six diets formulated for the feeding trial are presented in table 1. Similar observations were made for lipids and other nutrient parameters considered. Slight variations however occurred in the crude protein content of the formulated diets on chemically analysis and this may be due to differences in the by-products compositions. Values of their crude protein ranged between 39.86% and 40.20%. Similar observations were made for lipids and other nutrient parameters considered. Table 2 revealed the extent of utilization of the six diets by fish as well as their growth performance.

Highest mean weight gain (MWG) was recorded for fish fed 0% and 20% LSM based-diets which were significantly ($p < 0.05$) higher than values of other LSM inclusions while 40% and 60% LSM based diet were only marginally different ($P > 0.05$). MWG of fish

Table 1
Diet formulation and proximate composition of LSM based-diets in replacement of SBM at 0% - 100%

Gross composition ingredients (g/100 g/DM)	diet 1	diet 2	diet 3	diet 4	diet 5	diet 6
	0%	20%	40%	60%	80%	100%
SBM	23.39	19.51	14.64	9.75	4.88	-
LSM	-	4.88	9.75	14.64	19.51	24.39
Fishmeal	18.29	18.29	18.29	18.29	18.29	18.29
Groundnut cake	26.59	26.59	26.59	26.59	26.59	26.59
Yellow maize	27.38	27.38	27.38	27.38	27.38	27.38
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	1.35	0.25	0.25	0.25	0.25	0.25
Palm oil	0.75	0.75	0.75	0.75	0.75	0.75
Mineral /vit. supplements*	0.60	0.60	0.60	0.60	0.60	0.60
Chemical composition (%)						
Crude protein	40.14	40.02	39.97	39.86	40.18	40.20
Lipid	9.93	10.27	9.98	10.01	9.87	10.36
Crude fiber	2.41	2.39	2.56	2.58	2.57	2.81
Ash	7.03	8.21	8.33	9.62	9.19	8.85
Nitrogen free extract	36.32	35.74	35.58	38.01	36.33	38.25
Moisture	4.92	5.66	5.62	5.17	4.84	5.03
Gross energy (kcal/kg)	2890.41	2954.66	3000.42	2889.85	3086.00	3031.29

Biomix fish vitamin/mineral providing per kg of diet at 5 kg per tonne inclusion: 20,000 iu, vitamin A, 200 iu, α-tocopherol acetate 400 mg, Ascorbic acid 100 mg, Vit. D3, 200 mg Vit E, 8 mg Vit k3, 20 mg Vit B1, 30 mg Vit B2, 12 mg Vit B6, 50 mg Pantothenic acid, 0.8 mg Biotin, 150 mg Niacin, 0.05 mg Vit B12, 4.0 mg Cobalt, 40 mg Iron, 5.0 mg Iodine, 30 mg Manganese, 4 mg Copper, 40 mg Zinc, 0.2 mg Selenium, 100 mg Lysine, 100 mg Methionine, 100 mg Anti-oxidant.
Legends:
SBM: Soya Bean Meal
LSM: Leucaena Seed Meal

Table 2
Growth and nutrient utilization of *C. gariepinus* fed different inclusions of LSM based-diets

	Treatments-LSM inclusion rates (%)						
Parameters	0	20	40	60	80	100	SEM
Initial mean weight (g)	5.21 ± 0.14	5.29 ± 0.14	5.09 ± 0.14	5.22 ± 0.14	5.20 ± 0.14	5.23 ± 0.14	-
Final mean weight (g)	13.16 ± 0.02	12.98 ± 0.17	11.94 ± 0.11	10.97± 0.08	9.87 ± 0.11	9.30 ± 0.10	-
MWG (g)	7.95[a]	7.69[a]	6.85[b]	5.75[b]	4.67[c]	4.07[c]	0.65
Feed intake (g)/fish	56.78[a]	57.51[a]	54.40[c]	49.98[d]	54.54[bc]	55.18[b]	1.08
PWG (%)	60.41[a]	59.24[a]	57.37[a]	52.42[b]	47.32[c]	43.76[c]	2.77
SGR (%/day)	0.36[b]	0.46[a]	0.33[b]	0.39[b]	0.25[c]	0.23[c]	0.04
PER	0.35[a]	0.33[a]	0.32[a]	0.29[a]	0.21[b]	0.18[b]	0.03
FCR	0.71[c]	0.75[c]	0.79[bc]	0.90[b]	1.17[a]	1.36[a]	0.11
Nm (x10)	56.48[a]	56.17[a]	52.66[b]	49.77[b]	46.33[c]	44.67[c]	2.02
NPU	88.10[a]	87.87[a]	82.46[b]	79.19[b]	72.18[c]	69.56[c]	3.20
Survival rate (%)	92	92	88	92	76	80	-

Means with the same superscript in the same row are not significantly different (p> 0.05).

continue to decrease with increase in the LSM inclusion rate as 80% LSM and 100% produce the least set of values (4.67 g and 4.07 g) respectively, which were also not significantly different. Specific growth rate (SGR) was significantly higher (P< 0.05) in fish fed (20% processed LSM) than all other treatment including control (0% processed LSM) which was almost the same with values of 40% and 60%processed LSM. Fish fed 80% and 100% LSM were only marginally different in values of specific growth rates (0.25%/day and 0.23%/day) respectively. Fish growth exhibited significant inverse correlation with inclusion rate of LSM in the diet formulated. MWG and SGR had -0.62 and -0.57 correlation coefficient (r) respectively while PER had -0.78 and FCR had -0.64 correlation. Results also showed that values of PER were only marginally different in fish fed 0% to 60%LSM while values of FCR were only marginally different in fish fed 0% to 40% processed LSM. Nm and NPU produced results with similar trend. Diets with 0% and 20% LSM were statistically the same for both Nm and NPU and similarly for values of 40% and 60% LSM while values of 80% and 100% LSM had the least set of values. Survival rate was jointly highest in 0%, 20% and 60% LSM (92%) while 80% LSM had the least survival rate (76%).

Effects of the physiological changes in fish fed graded levels of LSM based diets for 112 day was presented in table 3. Fish carcass protein increased in all diets. Initial carcass protein value was 62.08% and this increased throughout in all the test diets. Fish carcass lipid seems to increase in all LSM with exception in 20% LSM where initial value was reduced from 4.27% to 4.12%.

Discussion

The growth and nutrient utilization by fish decreased as level of LSM inclusion increases in the diets. This observed pattern could probably be as a result of persistent consumption of leucaena meals which could retard animal growth rate as reported by Jones (12) and further buttressed by Tangendijaja (23) who recorded progressive depressed growth rate in rabbit fed increasing graded levels of leucaena leaf meal based-diet. Similar result on PWG and SGR could also be due to differences in the LSM inclusion which decreased at increasing level of LSM in the diets. Protein efficiency ratio (PER) was highest in fish fed 0% LSM but did not differ statistically (p> 0.05) from values of 20%, 40% and 60% LSM inclusions. These results seem to have direct link with feed intake. The importance of feed intake by fish as a determinant of fish performance has been strongly emphasized (7, 17, 18) while other studies (2, 13) pointed out the possibility of protein sparing effects by other nutrients in a feed, that is as more energy was supplied for metabolism through other nutrients, more protein intake is available for fish growth and tissue development. All diets produced higher values of fish carcass protein and lipid than initial values, yet there existed marginal difference among them indicating different utilization levels of the diets. These relatively high values of crude protein could be viewed alongside the work of Alegbeleye *et al.* (1) who reported that effective utilization of bambara groundnut at varying rates was responsible for variations in *Heteroclarias* carcass protein and lipid. This characteristic feed utilization efficiencies and consequents growth rates has been attribute to dietary protein quality (4, 21). The non-detection of crude fiber in the fish carcass composition was the same in all treatments and this had been said to be associated with effective utilization of diets according to Sotolu (22). Observed differences in the hematology of fish especially at significant level (P< 0.05) between 20% LSM fed fish and those of higher LSM inclusions could be as a result of the residual effect of mimosine present in the seeds after processing.

Conclusion

Improvement in the nutritional quality of *Leucaena leucocephala* seed meal was achieved by soaking in water. The crude protein content was enhanced and became readily acceptable by fish. Utilization of LSM by fish when processed by soaking in water was better at 20% inclusion level than at higher inclusion rates. However, there is still need for further studies towards increasing the utilization of LSM to 40% inclusion as

Table 3

Carcass Composition of *C. gariepinus* Fed LSM Based-Diets for 112 days

Parameters (%)	Initial	Final values at Different LSM inclusion rates (%)						
	Value	0	20	40	60	80	100	SE Mean
Crude protein	62.08	68.47	68.13	66.81	67.91	66.74	66.48	1.74
Lipid	4.27	5.93	4.12	5.98	4.53	4.96	5.58	0.19
Ash	11.60	11.14	10.83	10.94	11.02	10.86	10.80	0.50
Crude fiber	ND	ND	ND	ND	ND	ND	ND	-
NFE	5.23	2.47	3.02	3.01	2.78	2.82	3.94	0.29

ND – Not Detected
NFE: Nitrogen Free Extract

Means with the same superscript in the same row are not significantly different (p> 0.05).

most utilization assessed in this study were worst than those of 20% inclusion. Since weight gain of fish is what would translate into income for the fish farmer at the end of the production cycle, 20% inclusion rate of LSM in catfish diet would produce better and profitable result at present. Cost of fish production is expected to further reduce if more soya bean meal could be replaced by leucaena seed meal.

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