


Growth performance and haematology of *Clarias gariepinus* (Burchell, 1822) fed varying inclusions of *Leucaena leucocephala* seed meal based-diets

Evaluación del crecimiento y hematología de *Clarias gariepinus* (Burchell, 1822) alimentados con diferentes inclusiones de dietas basadas en harinas de semillas de *Leucaena leucocephala*

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ABSTRACT

Utilization of plant protein sources in aquaculture have continued to produce more promising results towards alleviating high cost of feeding. This study examined the utilization of *Leucaena leucocephala* 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. Solid wastes were siphoned everyday before feeding while total water exchange with fresh clean water was done when data on fish growth and haematology were collected. Chemical analysis of diets and feeds were carried out before experiment and that of fish alone was repeated after the experiment. Data from the completely randomized experiment were subjected to ANOVA and correlation analysis and L.S.D. 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 in fish diet $r = -0.62, -0.57, -0.78$ and -0.64 respectively. Fish carcass protein, packed cell volume and haemoglobin counts 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.

Key words: Plant protein, leucaena seed meal, soya bean meal, fish feeding, aquaculture

RESUMEN

La utilización de fuentes de proteínas vegetales en la acuicultura ha continuado para producir más resultados prometedores para aliviar el alto costo de la alimentación. Este estudio examinó la utilización de harina de semillas de *Leucaena* (HSL) para la producción sustentable de peces. Se formularon 6 dietas isonitrogénicas, dietas con 40% de proteína cruda donde HSL reemplazó a la harina de soya (HS) a 0, 20, 40, 60, 80 y 100% de las inclusiones. Alevines de bagre ($5,21 \pm 0,14$ g) almacenados en tanques de 25 peces/70 litros se alimentaron con dietas en triplicados, tres veces diariamente durante 112 días. Los desechos sólidos se sifonearon todos los días antes de la alimentación mientras el cambio total de agua con agua fresca y limpia se realizó cada dos semanas cuando se colectaron los datos del crecimiento de los peces y los caracteres hematológicos. Se llevaron a cabo análisis químico de las dietas y los alimentos antes del experimento y el análisis sólo de los peces se repitió después del experimento utilizando el método de la AOAC (2000). Se sometieron los datos del experimento completamente aleatorizado a ANOVA y análisis de correlación y la prueba de MDS se usó para separar los tratamientos a un 5% de probabilidad de acuerdo a Steel *et al.* (1997). La ganancia promedio de peso (GPP) y la tasa de crecimiento específico (TCE) de los peces alimentados con 20% HSL fueron estadísticamente diferentes ($p < 0,05$) de aquellos peces alimentados con HSL a tasas más altas de inclusión. GPP, TCE, PER y FCR significativamente ($p < 0,05$) se correlacionaron con las tasas de inclusión de HSL en la dieta de los peces, $r = -0,62, -0,57, -0,78$ y $-0,64$, respectivamente. La proteína en el canal de los peces, Volumen de células empaquetadas y conteos de hemoglobina de los peces fueron estadísticamente similares para peces alimentados con 0, 20 y 40% HSL LSM. La harina de semillas de *Leucaena* ofrece buenos valores nutritivos en la dieta de peces y su utilización fue mejor a 20% de inclusión para la producción sustentable de peces.

Palabras clave: Proteína vegetal, harina de semillas de *Leucaena*, harina de soya, alimentación de peces, acuicultura

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 (Ogunji *et al.*, 2005; F.A.O 2006). 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 (Hasting, 1976; Nwanna *et al.*, 2008). 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 (Pillay, 1990; Francis *et al.*, 2001). The need for such recommendations have been due to the presence of certain limiting factors in those ingredients such as high crude fibre content (Nwanna *et al.*, 2008), antinutritional factors such as in *Vigna subtarreana* (Alegbeleye *et al.*, 2001). 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 (Francis *et al.*, 2001; Cho, *et al.*, 1974).

Leucaena leucocephala demonstrated good potential to serve as a useful plant protein source in fish ration and in the livestock industry generally (Jones, 1979; Ter Muelen *et al.*, 1981; D'Mello and Acamovic, 1989). However, it has been established that leucaena contain mimosine-a non-protein amino acid capable of inhibiting protein biosynthesis in animal causing growth retardation if consumed intensively (Ter Muelen *et al.*, 1981; D'Mello and Acamovic, 1989). Cruz and Laudencia (1977) found out that 33 to 100% leucaena leaf meal as integral part of supplemental feed enhanced the growth of *Oreochromis niloticus* fingerlings in Lake Laguna. D'Mello and Acamovic (1989) on the other hand, stressed its potential as a good feed ingredient in the culture of mollies and topminnows (*Poecilia spp*) and freshwater prawn (*Macrobrachium rosenbergii*). A preliminary study by Sotolu and Faturoti (2008) 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. This study examined the utilization of *L. leucocephala* seed meal (LSM) for sustainable fish production

MATERIALS 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 (1987). 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-PLSM) were chemically analyzed according to A.O.A.C (2000). Parameters of importance include crude protein, crude fibre, fat, ash, moisture and calorific value. Six isonitrogenous diets were prepared (40% C.P) 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 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 (2000) while that of the experimental fish alone was repeated at the end of the experiment. Water quality parameters (pH, Dissolved oxygen, ammonia) were kept favorable for fish by constant renewal of water in fish tanks at a flow rate of 2.5l/min till the end of the experiment. Catfish fingerlings (5.21±0.14g) were stocked in a circular tank of 70 liter capacity 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 09:00h-09:30h, 14:00h-14:30h and 19:00h-19:30h 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), Gross energy was calculated according to Jobling (1983) with multiplier factors of carbohydrate, 4.1 kcal/g, protein, 5.4kcal/g and lipids, 9.5kcal/g.

$$\text{SGR} = \frac{(\text{Loge final weight} - \text{Loge initial weight})}{\text{Culture period}} \times 100$$

$$\text{FCR} = \frac{\text{Total feed fed}}{\text{Total wet weight gain}}$$

$$\text{PER} = \frac{\text{Wet weight gain}}{\text{Amount of protein fed}}$$

$$\text{Nm} = \frac{0.549 (a+b) h}{2}$$

Where,

a = Initial mean weight of fish

b= final mean weight of fish

h= experimental period in days

$$\text{NPU} = \frac{(\text{N content of fish after experiment} - \text{N content of fish before experiment} + \text{N metabolism})}{\text{N of experimental diet}}$$

$$\text{Survival rate (SR)} = \frac{(\text{Number of fish stocked} - \text{Mortality})}{\text{Number of fish stocked}} \times 100$$

Fish were tranquilized with 150mg/L of Tricane methane Sulphonate for blood collection according to Schalm *et al.* (1975). Initial fish blood samples were collected before feeding trial that is 0th day with the aid of needle and syringe into bottles with EDTA as anticoagulant. Subsequent blood samples were collected on 42nd, 56th, 70th, 84th, 98th and 112th day as the final collection. Blood samples collected were immediately taken to the laboratory for haematological analysis on each occasion as described by Schalm *et al.* (1975).

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 level according to Steel *et al.*, (1997).

RESULTS

Proximate composition of processed and unprocessed leucaena seeds is given in Table 1. Processing increased protein content from 22.7 to 36% while crude fiber decreased from 11.4 to 7.1 in the unprocessed and processed leucaena respectively. Diet formulation gross and proximate compositions of the six diets formulated for the feeding trial are presented in Table 2. Values for crude protein content ranged between 39.86% to 40.20% while crude lipid ranged from 9.87 to 10.36%. Growth performance feed and protein utilization is given in Table 3. Highest mean weight gain (MWG) was recorded for fish fed 0% and 20% LSM based-diets 7.9g and 7.7g respectively which were significantly ($p < 0.05$) higher than the respective values of the other LSM inclusions while 40% and 60% LSM based diet were only marginally different ($P < 0.05$). MWG of fish

Table 1. Proximate and mineral composition of unprocessed (ULSM) and processed leucaena seed meal (PLSM).

LSM	Crude protein (%)	Crude fibre (%)	Fat (%)	Ash (%)	Moisture (%)	Energy kcal/ kg	Na (%)	K (%)	P (%)	Ca (%)	Mg (%)
ULSM	22.75	11.38	6.12	5.98	15.14	2833.50	0.01	1.62	0.46	0.41	0.22
PLSM	36.01	7.11	5.18	3.74	12.56	2899.76	0.03	1.68	0.68	0.42	0.29

continue to decrease with increase in the LSM inclusion rate as 80% LSM and 100% produce the least set of values (4.67g and 4.07g) respectively, which were also not significantly different. Specific growth rate (SGR) was significantly higher ($P<0.05$) in fish fed (20%LSM) than all other treatment including control (0%LSM) which was almost the same with those of 40% and 60%LSM. Fish fed 80%

and 100% LSM were only marginally different 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 a -0.62 and -0.57 correlation coefficients (r) respectively while PER and FCR had -0.78 and -0.64, respectively (data not shown).

Table 2. Diet formulation and proximate composition of LSM based-diets in replacement of SBM at 0% - 100%).

Gross composition ingredients (g/100g/DM)	Diet 1 0%	Diet 2 20%	Diet 3 40%	Diet 4 60%	Diet 5 80%	Diet 6 100%
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 (%) on a dry matter basis						
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 fibre	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
NFE	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 5kg per tonne inclusion: 20,000 iu, vitamin A, 200 i.u, α -tocopherol acetate 400mg, Ascorbic acid 100mg, Vit. D3, 200 mg Vit E, 8 mg Vit k3, 20mg Vit B1, 30 mg Vit B2, 12 mg Vit B6, 50 mg Pantothenic acid, 0.8 mg Biotin, 150 mg Niacin, 0.05mg Vit B12, 4.0mg 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.

Table 3. Growth and nutrient utilization of *Clarias gariepinus* fed different inclusions of LSM based-diets.

Parameters	Treatments-LSM inclusion rates (%)						SEM
	0	20	40	60	80	100	
Initial mean weight (g)	5.21 \pm 0.14	5.29 \pm 0.14	5.09 \pm 0.14	5.22 \pm 0.14	5.20 \pm 0.14	5.23 \pm 0.14	-
Final mean weight (g)	13.16 \pm 0.02	12.98 \pm 0.17	11.94 \pm 0.11	10.97 \pm 0.08	9.87 \pm 0.11	9.30 \pm 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 ($p<0.05$)

Results also showed that values of PER were only marginally different in fish fed 0% to 60%LSM (0.35 to 0.29) while values of FCR were only marginally different in fish fed 0% to 40%LSM (0.71 to 0.79). In both parameters examined further increase of LSM in the diet (60 and 100%) resulted in significant worsening. Protein utilization expressed by Nm and NPU in fish decreased significantly from 40% LSM inclusion level. Values of 0% and 20% LSM were statistically the same and similarly values of 40% and 60% LSM fed fish, while values of 80% and 100% LSM had the lowest set of values. Survival rate was jointly highest in 0%, 20% and 60% LSM (92%) while 80% LSM had the least survival rate (76%).

The effects on the physiological changes in fish fed graded levels of LSM based diets for 112 day is presented in Table 4. Fish carcass protein increased throughout in all diets. Initial carcass protein value was 62.08% while 0% LSM produced 68.47% C.P and 100% LSM produced 66.48% C.P respectively. Fish carcass lipid seems to increase in all LSMs with exception in 20% LSM where initial value was reduced from 4.27% to 4.12%.

Fish PCV increased from initial 28.21% to 28.22% in 20% LSM and subsequently decreased marginally to 28.16% in 40% LSM. The least PCV value was recorded for 100% LSM (27.99%) which was not significantly different from 80% LSM (28.02%). Results of Hb also increased from its initial value but up to value of 40% LSM where differences were only marginal ($P < 0.05$). Rbc increased in value from initial $2.36 \times 10^6/\text{ml}$ to $2.49 \times 10^6/\text{ml}$ in 0% LSM. Rbc was not significantly different in values for

initial composition, 40%, 60% and 80% LSM while 100 LSM produced significant least value. Wbc did not vary significantly from initial value to 20% LSM inclusion but there was significant variation ($P < 0.05$) between 40%, 60% and 80% LSM while 100% LSM was only marginally different from 40% LSM. The highest value for MCV was found in fish fed 100% LSM while the least was recorded for fish fed 0% LSM with significant variation ($P < 0.05$) while initial value 119.53(fl) was only marginally different from the value of 60% LSM (121.20 fl).

DISCUSSION

Growth and nutrient utilization by fish decreased as level of LSM inclusion increases in the diets. This observed pattern could probably be a result of persistent consumption of leucaena meals which could retard animal growth rate as reported by Jones (1979) and further buttressed by Tangendijaja *et al.*, (1990) who recorded progressive depressed growth rate in rabbit fed increasing graded levels of leucaena leaf meal based-diet. Similarly the results on PWG and SGR could also be due to differences in the LSM inclusion which decreased at increasing level of LSM in the diets. The consumption of antinutrient factors contained in the LSM based diets (mimosine like) are probably responsible for retarded growth response of the fish. 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 (Preston and Leng, 1987; Faturoti, 1989; Pillay 1990) while other studies (Anderson *et al.*,

Table 4. Carcass and haematology composition of *Clarias gariepinus* fed LSM based-diets for 112 days.

Parameters (%)	Initial Value	Final values at Different LSM inclusion rates, %						SE mean
		0	20	40	60	80	100	
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 fibre	ND	ND	ND	ND	ND	ND	ND	-
NFE	5.23	2.47	3.02	3.01	2.78	2.82	3.94	0.29
Haematocrits								
PCV (%)	28.21 ^a	28.22 ^a	28.20 ^a	29.16 ^b	28.15 ^b	28.02 ^c	27.99 ^c	0.06
Hb (gm/100ml)	8.39 ^a	8.43 ^a	8.41 ^a	8.37 ^a	8.22 ^b	8.20 ^b	8.17 ^b	0.05
Rbc ($\times 10^6/\text{ml}$)	2.36 ^b	2.49 ^a	2.44 ^a	2.38 ^b	2.34 ^b	2.31 ^b	2.24 ^c	0.05
Wbc ($\times 10^3/\text{ml}$)	16.17 ^a	16.13 ^a	16.16 ^a	16.20 ^a	16.29 ^b	16.39 ^c	16.21 ^a	0.04
MCV (fl)	119.53 ^b	113.44 ^c	114.23 ^c	115.98 ^c	121.20 ^b	127.29 ^a	130.37 ^a	2.46

ND – Not Detected. Means with the same superscript in the same row are not significantly different ($p < 0.05$)

1984; Keembiyehetty and De-Silva, 1993) 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 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.*, 2001) who reported that effective utilization of bambara groundnut at varying rates was responsible for variations in *Heteroclaris* carcass protein and lipid. 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, 2008). 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. This was similar to the reports of Adeyemo (2005) and Osuigwe *et al.*, (2005) that ascertained the reduction in values of haematological parameters such as PCV, Hb and Rbc were due to the presence of toxic substances in the diet of fish. It could therefore be inferred from this study that fish with lower LSM inclusion levels were of better health status than those of higher LSM inclusions based on the earlier submissions of Svobodova *et al.*, (1991), Alegbeleye (2005) and Ochang *et al.*, (2007).

CONCLUSION

The utilization of LSM by fish when processed by soaking in water was better at 20% inclusion level than at higher inclusion rates. 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. However, there is still need for further studies towards increasing the utilization level of LSM to 40% inclusion as most utilization and haematological parameters assessed in this study were marginally different from those of 20% inclusion. 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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