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GROWTH PERFORMANCE AND HEMATOLOGICAL EFFECTS OF VARYING DIETARY PROCESSED *LEUCAENA LEUCOCEPHALA* SEED MEAL IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) JUVENILES

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ABSTRACT

This study investigated growth performance and hematological effects of varying dietary processed *Leucaena leucocephala* seed meal (LSM) in *Clarias gariepinus* (Burchell, 1822) juveniles. Seeds of *L. leucocephala* were processed by soaking in water for 72 hours in order to improve their nutritional quality as a protein source for aquaculture. Five isonitrogenous diets (40% crude protein) containing different dietary levels of processed leucaena seed meals (LSM) at 0, 25, 50, 75 and 100% proportions of fishmeal were fed to *Clarias gariepinus* juveniles (21.25 ± 1.18 g average weight) for 70 days. The processed leucaena seeds and the five diets prepared were analyzed for their proximate composition. Data were collected on fish growth, feed consumption pattern and haematology. At the end of the experiment, dietary leucaena seed meal produced positive growth effect in fish under all the treatments. Growth performance and feed utilization efficiency of fish fed dietary LSM was inferior with increasing LSM inclusions as weight gain reduced from 26.49 ± 0.62g in diet 1 (0% LSM) to 20.25 ± 0.10g in diet 5 (100% LSM). There was, however, no significant difference between the weights gains, protein efficiency ratio (PER), feed conversion ratio (FCR) and specific growth rate (SGR) of fish fed on 0% and 25% LSM diets ($p>0.05$). Haematology of experimental fish also was significantly inferior ($p<0.05$) at higher dietary LSM inclusion especially at values from 50% inclusion and above. The superior growth performance, nutrient utilization and haematology status of experimental fish at lower LSM inclusion levels (0 and 25%) could be as a result of higher protein quality inferred through the estimation of PER, which can be attributed to well balanced amino acids constituents of fishmeal. Inclusion of LSM at 25% in *Clarias gariepinus* (catfish) diet produced the best growth rate while the 50% inclusion rate requires further study in order to increase its utilization efficiency in fish production.

Key words: Leucaena, fishmeal, protein, inclusions, aquaculture

INTRODUCTION

Aquacultural production in the developing world is greatly constrained by undersupply, scarcity and high cost of conventional fish feed [1]. The ever growing cost and uncertainties about the quality and availability of fishmeal have also compelled most aquaculture nutritionists and feed manufactures to use cheap and readily available plant protein materials (such as *Leucaena leucocephala*) as an alternative to the more costly fishmeal [2, 3]. *Leucaena leucocephala* has been described as a fast growing and drought resistant tropical leguminous tree, which offers the widest assortment of uses among other legumes [4, 5]. Improved growth responses of *Oreochromis mossambicus* and *O. niloticus* fed on diets containing 100% leucaena leaf meal (LLM) have been reported [6]. On the other hand, very poor growth response has been observed in *O. mossambicus* fed on fishmeal diets in which leucaena leaf meal constituted 25% of the meal [7]. A trend of reduced growth performance and feed utilization efficiency was reported in Nile tilapia fed on increasing dietary leucaena leaf meal [8]. There is information that fish fed on processed *L. leucocephala* seed meal had high digestibility value and high survival rate Hecth *et al*; Sotolu and Faturoti [9, 10]. Besides its use in production of fish meal, leucaena has been reported as a valuable protein food for ruminant animals [11] and is also used in monogastric production [12, 13]. *Clarias gariepinus* is the most widely cultured fish species in Nigeria [1]. This could be as a result of its ability to withstand stress due to handling and as a result of unfavourable environmental conditions. It is known to have high conversion rate of feed of diverse substances ranging from plant materials, agricultural by-products and industrial wastes [1]. The fish is presently in high demand in Nigeria; hence there is the need to increase its production at minimum cost towards ensuring the supply of sufficient food fish to the populace at affordable prices. This study, therefore, investigated growth performance and hematological effects of varying dietary processed *Leucaena leucocephala* seed meal (LSM) in *Clarias gariepinus* juveniles as the basis for its use as a cheap alternative to fishmeal.

MATERIALS AND METHODS

Experimental design

The design of the experiment was a Completely Randomized Design (CRD).

Preparation of experimental diets

Seeds of leucaena were processed by soaking in water 72 hours [14] and later chemically analyzed. The processed leucaena seed meal (PLSM) was used to formulate 5 isontrogenous (40% CP) catfish diets replacing fishmeal at 0, 25, 50, 75 and 100% inclusion level. All test diets were chemically analyzed for crude protein, crude fibre, fat, ash, moisture, nitrogen free-extract and calorific value using standard methods [15]. The experiment was set up in the postgraduate laboratory section of the Department of Wildlife and Fisheries Management University of Ibadan. A total of 150 juveniles of *Clarias gariepinus* (average weight of 21.25 ± 1.18 g) were randomly distributed into the five treatments in triplicates. Each experimental tank of 70 litres capacity was covered tightly with mosquito net on the top to prevent fish from jumping out throughout the 70 days experimental period. Fish were fed twice daily *ad libitum* and quantities of feed consumed were recorded daily. Water quality parameters which included dissolved oxygen, pH and temperature were monitored and maintained constant throughout the duration of the experiment by regular renewal of the water. To achieve this, water in each experimental tank was reduced to half every morning at 08.00 h before feeding and total water exchange was done fortnightly when data were taken on fish growth and feed utilization efficiency.

Determination of fish growth and performance

Fish growth and performance were determined as follows:

1. Mean weight gain (MWG) in grams = W_2 - W_1
2. Weight gain (MWG) = $W_2 – W_1$
3. Specific Growth Rate (SGR) = (Log W_2 – Log $W_1/T_2 – T_1$) x 100
   Where: W_2 = final weight of fish, W_1 = initial weight (g) of fish, T_2 = end of experiment and T_1 = beginning of experiment (days)
4. Protein efficiency ratio (PER) = weight gain (g)/Protein intake (g)
5. Feed conversion Ratio (FCR) = Total feed intake/Weight gain (g)
6. Protein intake = Feed fed x crude protein of the feed.
7. Survival rate (%) = (Initial no. of fish stocked – mortality)/Initial no. of fish x 100.

Haematological study

Fish haematology was carried out using standard procedures [16, 17]. Initial blood samples were taken prior to the commencement of the feeding and final blood samples were taken from fish in triplicates on the last day of the experiment. The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglotan (MCH) were calculated using the total red blood cell count (RBC), haemoglobin concentration (Hb) and haematocrit (Hct) [18].

MCHC (%) = (Hb/PCV) x 10
MCH (pg) = (Hb/RBC) x 10
MCV (fl) = PCV/RBC x 100

Statistical analysis

All data collected during the experiment were subjected to the one-way analysis of variance (ANOVA) using the SPSS version 10.0 for windows and significant mean differences were separated at 5% [19].

RESULTS

The proximate analysis of processed and unprocessed leucaena seed meals (LSMs) revealed that processing increased the value of crude protein and a decrease in crude fibre content. There was an increase by 13.26% for crude protein content and a decrease by 4.27% for crude fibre content from ULSM to PLSM, respectively (Table 1). Crude fat contents were 5.18% and 6.12% for PLSM and ULSM, respectively. The crude protein contents (40.02 - 40.21%) of the experimental diets were similar with crude protein in processed leucaena seed meals (LSMs) (Table 2). Crude fat, fibre, ash and energy contents were also comparable among the different diets. However, the diet with 75% LSM was highest in crude fat and crude fibre contents. Diet with 50% LSM inclusion had the least crude fat content while that with 100% LSM was the poorest in crude fibre content. Results indicate that LSM based diets were adequately consumed by experimental fish. Feed consumed ranged from 34.16 ± 1.10 g to 39.30 ± 1.05 g and average survival rate was 100% in each treatment. At the end of the 70th day of feeding experimental trials, fish exhibited significant weight gain, specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion ratio (FCR). Fish fed on 0% and 25% LSM had superior weight gains, PER and FCR over other treatments ($p<0.05$). However, there was no significant difference ($p>0.05$) between the SGRs of the two treatments (0% and 25% LSM treated diet) and that of 50% LSM treated diet (Table 3). For all the parameters assessed, there were no significant differences between fish under treatment 4 and 5 (75% and 100% LSM).

The results of haematological characteristics of fish are presented in Table 4. Haematological changes among the treatments included: anaemia marked by significantly low ($p<0.05$) Hb, PCV and RBC in fish fed diets that contained 50%, 75% and 100% LSMs. Mean cell volume values were significantly high in all experimental diets except the 25% LSM diet, which was only marginally different from initial value. Values of MCH and MCHC were all statistically similar for fish fed on 0% to 50% LSM based-diets in relation to the initial values while values for 75 and 100% LSM based-diets were significantly different. Total white blood cell (WBC) count was marginally different among treatment with 0 – 50% LSM diets compared with initial value but values of diets with 75 and 100% LSM were significantly higher ($p<0.05$) than initial value of WBC. There were also significant differences among most of the haematological parameters assessed between fish fed 75% and 100% LSM based-diets.

DISCUSSION

Presently, fishmeal constitutes a substantial part of formulated feed for diverse fish species and livestock globally [20]. In the present study, the replacement of fishmeal in parts by LSM produced good formulated feeds with low differential crude fat, fibre and ash contents compared with the control diet. Improvement in the nutritional quality of the seed meal compared with the raw form of leucaena leaf meal from the present study is similar to the previous reported observations [8]. Improved performance of LLM in fish production after soaking of the seeds in water for 2 days has also been reported [21, 22] to be useful for removing of up to 90% of mimosine (antinutitional factor) present in the seeds. As a result of these improvements in the nutritional values of the LLSM, 100% survival rate was recorded in all treatment, which is contrary to previous observations [11, 23]. However, reduced weight gain, SGR, PER and higher FCR values at higher LSM proportions (> 50%) in fishmeal diet indicated poor utilization of LSM by fish. It is well known that the efficiency of feed utilization and consequent growth performance is affected by the quality of dietary protein [24, 25]. Protein quality is usually determined by its ability to support growth and development of the animal, which in turn depends primarily on the composition of essential amino acids. In this study, protein quality was indicated by direct estimation of PER. The lowest PER (hence lower protein quality) at 100% LSM inclusion in the fish meal diet could probably be attributed to significantly lower weight gain and SGR recorded.

The marginally different values of SGR at 50% LSM inclusion compared with the control and 25% inclusion level indicated that fish can utilize LSM to attain a similar growth pattern without significant difference as the control. This observation could be related to an earlier report [26], which recommends 40% inclusion level of LLM in fishmeal diets. Haematology is used as an index of fish health status in other to detected physiological changes due to unfavourable condition such as exposure to disease and metal pollutants [27]. The reduction in haemoglobin (Hb), Pack cell volume (PCV) and the subsequent variation in MCH values at 75 and 100% LSM inclusions was perhaps, an indication of anaemia resulting from shrunk red blood cell- a situation that can result in fish asphyxiation and death. This observation is similar to earlier documented reports [28]. Values of mean cell haemoglobin concentration (MCHC) followed the same trend as those of MCH, which further established the negative effect of reduction in Red blood cell count (RBC) and the resultant increase in values of WBC with increasing LSM inclusions. The negative health status was again significant ($p<0.05$) at 75 and 100% LSM inclusions. This inverse relationship is similar to the previous observations [28, 29]. Superior haematological values found in the present study are also within the recommended values [30].

CONCLUSION

Among the individual dietary LSM inclusions tested in the present study, 75% and 100% inclusion levels are poorly utilized by fish given the least values of growth and health status indices. The inclusion of the test ingredient (LSM) at 50% is, however, encouraging due to its marginally different SGR value from the control diet and some of the haematological parameters assessed. Apart from the best inclusion level (25% LSM) in the present study, the 50% inclusion level could probably produce similarly improved fish performance if fortified with essential amino acid, which seems to be its only limitation as compared to fishmeal that has well balanced amino acid profile.

Table 1: Proximate composition of processed leucaena seed meals (LSM)

LSM	Crude protein (%)	Crude fibre (%)	Fat (%)	Ash (%)	Moisture (%)	Energy (Kcal kg)
PLSM	36.01	7.11	5.18	3.74	12.56	2899.76
ULSM	22.75	11.38	6.12	5.98	15.14	2833.50

PLSM=Procesed Leucaena Seed Meal
UPLSM=Unprocessed Leucaena Seed Meal

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Table 2: Gross ingredients and proximate composition of experimental diets

	LSM Inclusions				
Ingredient (g/100g/DM)	**0%**	**25%**	**50%**	**75%**	**100%**
Fishmeal	28.30	21.23	14.15	7.08	-
LSM	-	13.49	26.99	40.48	53.99
SBM	19.16	19.16	19.16	19.16	19.16
GNC	18.57	16.42	14.17	12.46	10.56
Maize	28.27	24.00	20.37	15.12	10.59
D-calcium phosphate	1.50	1.50	1.50	1.50	1.50
Fish premix*	1.00	1.00	1.00	1.00	1.00
Methionine + Lysine	2.00	2.00	2.00	2.00	2.00
Palm oil	0.70	0.70	0.70	0.70	0.70
Proximate composition (g/10g/DM)					
Crude protein (%)	40.02	40.21	40.20	40.17	40.21
Crude fat (%)	11.19	11.38	10.63	13.10	11.26
Crude fibre (%)	5.21	4.66	5.27	5.63	5.40
Ash (%)	12.16	11.83	11.80	12.02	12.16
Ether extract (%)	31.42	31.92	32.10	29.08	30.97
Gross energy (Kcal/g/DM)	431.64	442.15	438.26	436.16	430.45

LSM = Leucaena seed meal
SBM = Soyabean meal
GNC = Groundnut cake
*Biomix fish vitamin/mineral providing per kg of diet at 5kg per tonne inclusion: 20,000 iu, vitamin A, 200 i.u, 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, 160mg Vit. C, 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.

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Table 3: Growth and nutrient utilization of *C. gariepinus* fed dietary LSM inclusions

	Treatment (LSM inclusion)				
Indices	**0%**	**25%**	**50%**	**75%**	**100%**
Initial weight (g)	21.25	20.17	20.12	21.16	20.16
Final weight (g)	47.74	46.92	44.17	42.28	40.41
Weight gain(g)	26.49±0.62[a]	26.75±1.20[a]	24.05±0.13[b]	21.12±0.32[c]	20.25±0.10[c]
Feed consumed	34.16±1.10[c]	33.85±1.02[c]	36.34±0.76[b]	38.16±0.32[a]	39.30±1.05[a]
SGR	0.50±0.02[a]	0.52±0.12[a]	0.49±0.11[a]	0.43±0.04[b]	0.43±0.14[b]
PER	1.94±0.19[a]	1.97±0.33[a]	1.65±0.26[b]	1.38±0.12[c]	1.28±0.31[c]
FCR	1.29±0.05[c]	1.27±0.01[c]	1.51±0.10[b]	1.80±0.07[a]	1.94±0.11[a]
Survival rate (%)	100	100	100	100	100

Mean values on the same row with different superscripts are significantly different ($p<0.05$).

Table 4: Haematological indices of *C. gariepinus* fed dietary LSM inclusion

	LSM inclusions					
Parameters	**Initial**	**0%**	**25%**	**50%**	**75%**	**100%**
Hb (%)	9.12±0.36[a]	8.41±0.15[a]	8.43±.12[a]	7.06±0.11[b]	7.50±1.22[b]	7.43±0.13[b]
PCV (%)	24.83±2.16[a]	24.66±0.75[a]	22.10±0.23[b]	21.23±1.24[b]	18.47±0.25[c]	13.00±0.41[d]
ESR (mm/h)	10.85±1.30[b]	11.26±1.41[b]	11.19±0.12[b]	12.00±0.30[a]	11.10±0.26[b]	10.76±0.37[b]
WBC ($x10^3$/ul)	3.49±0.10[c]	3.56±0.24[bc]	3.58±0.12[bc]	3.66±0.31[bc]	3.82±0.41[ab]	4.14±0.06[a]
RBC ($x10^6$/ul)	3.57±0.02[a]	3.34±0.32[a]	3.17±0.16[a]	2.77±1.03[b]	2.47±0.12[b]	2.06±0.08[c]
MCH(pg)	25.55±1.20[c]	25.18±0.27[c]	26.48±0.46[c]	27.30±0.18[c]	30.36±0.24[b]	36.07±0.17[a]
MCV(fl)	69.65±1.73[c]	73.83±2.33[b]	71.97±2.06[bc]	76.64±2.42[a]	74.78±1.27[a]	63.11±1.26[d]
MCHC (%)	36.73±0.86[c]	34.10±1.18[c]	36.78±2.03[c]	35.16±2.50[c]	40.61±1.24[b]	57.15±2.15[a]

Mean values on the same row with different superscripts are significantly different ($p<0.05$).

REFERENCES

1. **Fagbenro OA and IA Arowosegbe** Utilization of agricultural wastes and by-products in fish feeds production in Nigeria. *Proc. 6th* Ann. FISON Conf., Lagos 1991: 121-130.

2. **Lim C and W Dominy** American Soybean Association. Utilization of plant proteins by warm-water fish. USDA-ARS, Tropical Aquaculture Research Unit; The Oceanic Institute, Hawaii, 1989.

3. **New MB and UN Wijkstrom** Use of fishmeal and fish oil in aquafeeds: Further thoughts on the fishmeal trap. Food and Agriculture Organization of the United Nations; F.A.O Fisheries Circular No. 975, 2002: Rome.

4. **NAS** (National Academy of Science). Leucaena: Promising Forage and Tree Crop for the Tropics. National Academy of Sciences; Washington, D.C. 1977: 187.

5. **D'Mello JPF and T Acamovic** *Leucaena leucocephala* in poultry nutrition–a review. *Anim. Feed Sci. Technol.* 1989; **26**:1-28.

6. **Pantastico JB and JP Baldia** Ipil-ipil leaf meal as supplement for *Tilapia nilotica* in cages. *Fish Res. J. Philipp*., 1980; **5** (**2**): 63-68.

7. **Cruz EM and LL Laudencia** Protein requirement of *Tilapia mossambica* fingerlings Kalikasan. *Phillp, J. Biol*., 1977; **6**: 177-182.

8. **Wee KL and SS Wang** Nutritive value of Leucaena leaf meal in pelleted feed for Nile tilapia. *Aquaculture* 1987; **62**:79-108 pp.

9. **Hecth T, Uys W and PJ Britz** The culture of Sharptooth Catfish, *Clarias gariepinus* in Southern African. South Africa National Scientific Programme Report No. 153. 1988: 47-61.

10. **Sotolu AO and EO Faturoti** Digestibility and Nutritional Values of Differently processed *Leucaena leucocephala* (Lam De Wit) Seed Meals in the Diet of African Catfish (*Clarias gariepinus*). *Middle-East J. Sci. Res*., 2008; **3** (**4**):190 – 199.

11. **Jones RJ** The value of *Leucaena leucocephala* as a feed for ruminants in the tropics, *W. Anim. Rev*. 1979; **31**:13-23.

12. **Labandan MM** Effects of various treatments and additives on the feeding value of ipil-ipil leaf meal in poultry. *Philipp. J. Agric*. 1988; **53**:392-401.

13. **Khadiga AA, Mohamed EA and HM Hasabelrasoul** The effects of dietary graded *Leucaena leucocephala* seeds on performance and blood parameters in broiler chicks. *Pak. J. Nut.* 2008; **7** (**2**): 385-390.

14. **Sotolu AO** Nutrient potentials of water hyacinth as a feed supplement in sustainable aquaculture. *Obeche* 2008; **26** (**1**): 45 – 51.

15. **Association of Official Analytical Chemists (AOAC)** Official Method of Analysis. *17th Edition*, Washington D.C. U.S.A, 2000.

16. **Svobodova Z, Fravda D and J Palakova** Unified methodsof haematological examination of fish. Research Institute of fish culture and hydrobiology, Vodnany Czechoslovekia, 1991: 331.

17. **Wagner EJ, Jeisen T, Arndt R, Routledge MD and Q Breddwisch** Effects of rearing density upon cutthroat trout hematology, hatchery performance, fin erosion and general health and condition. *The Prog. Fish-Cult.*, 1997; **59**:173-187.

18. **Dacie JV and SN Lewis** Practical Haematology. *9thEdition.* Edinburg, Churchill Livingstone, London 2001: 633.

19. **Steel RG, Torrie JH and DA Dickey** Principles and Procedures of Statistics, A Biometric Approach, 3rd edition. McGraw-Hill Companies Inc., New York, USA 1997: 121.

20. **FAO.** Food and Agriculture Organization. The State of World Fisheries and Aquaculture 1998; FAO Fisheries Department. Rome, Italy, 1999: 112.

21. **Francis G, Makkar HPS and K Becker** Anti-nutritional factors present in plant-derived alternative fish feed ingredients and their effects in fish. *Aquaculture* 2001; **199** (**3-4**): 197-228.

22. **Alceste CC and DE Jory** TILAPIA-Alternative protein sources in tilapia feed formulation. *Aquaculture Magazine* Jul/Aug 2000; **26** (**4**): 3pp.

23. **Ter Muelen U, Struck S, Schulke E and EA El-Harith** A review on the nutritive value and toxic aspects of *Leucaena leucocephala*. *Trop. Anim. Prod.* 1979; **4**:113-26.

24. **Tacon AGJ, Webster JL and CA Martinez** Use of solvent extracted sunflower seed meal in complete diets for fingerling of rainbow trout (*Salmo gairdneri*). *Aquaculture* 1984; **43**:381-389.

25. **De Silva SS and TA Anderson** Fish nutrition in aquaculture. London: Chapman and Hall, 1994: 254.

26. **Santiago CB, Aldaba MB, Laron MA and OS Reyes** Reproductive performance and growth of Nile tilapia *Oreochromis niloticus* broodstock fed diets containing *Leucaena leucocephala* leaf meal. *Aquaculture* 1988; **70** (**1-2**): 53-62.

27. **Duthie GG and L Tort** Effect of dorsal aortic cannulation on the respiration and haematology of the Mediterranean dogfish, *Scyliorhinus canicula*. *Comparative. Biochem. and Physiolo*. 1985; **81A:** 879-883.

28. **Adeyemo OK** Haematological and Histopathological effects of cassava mill effluent in *Clarias gariepinus*. *Afr. J. Biomed. Res.* 2005; **8**: 179-183.

29. **Subhadra B, Lochmann R, Rawles S and RR Chen** Effect of dietary lipid source on the growth, tissue composition and hematological parameters of largemouth bass (*Micropetrus salmonides*). *Aquaculture*, 2006; **255**:210-222.

30. **Blaxhall PC and KW Daisley** Routine haematological methods for use with fish blood. *J. Fish Biol*. 1973; **5**:771-781.