20

Utilization of *Moringa oleifera* Leaves and *Lannea barteri* Bark Extract As Growth Promoting Additives in the Diet Of *Clarias gariepinus* Fingerlings

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Abstract

An experiment was conducted to determine the effects of plant additives as fish growth promoter in *Clarias gariepinus* fingerlings. Five hundred and forty (540) *Clarias gariepinus* fingerlings (4.6 ± 0.02g) were distributed into 27 re-circulatory tanks of 50 litres capacity filled with 20 litres of freshwater. The fishes were distributed in replicate of 9 dietary treatments. The diets comprised of Non-Additive (NAD), Moringa Whole (MWL), Moringa Aqueous Extract (MAE), Moringa Ethanol Extract (MEE), Lannea Whole (LWL), Lannea Aqueous Extract (LAE), Lannea Ethanol Extract (LEE), Antibiotic (ANTB) and a Commercial Reference Diet (CRD) as control diet. The experiment revealed that, the survival rate ranges between 90 and 98% in which the highest survival rates were recorded in the group of fish that were fed plant additives. The growth parameters showed significance difference ($p<0.05$). However, there were no significance differences ($p>0.05$) in the growth parameters of fishes fed CRD, NAD and ANTB. Among the plant additives based diets, best growth was recorded in LWL based diet (9.077 ± 0.58). There were no significant differences ($p>0.05$) among CRD, NAD and ANTB in the Specific Growth Rate values (2.46 ± 0.21%/day, 2.48 ± 0.17%/day and 2.23 ± 0.23%/day) respectively. Moreover, there were no significant differences ($p>0.05$) for Feed Conversion Ratio while, the Protein Efficiency Ratio value in CRD, NAD, and ANTB were significantly higher ($p<0.05$) than MWL, MAE, MEE, LWL, LAE, LEE respectively. A significantly high Apparent Net Protein Utilization (ANPU) was recorded in LEE (98.33 ± 2.89) based diet while ANTB had (5.57 ± 1.92) which is the lowest ANPU value. From the research, Lannea whole incorporation in the diet of *Clarias gariepinus* had positive effect on its growth.

Keywords: Utilization, *Moringa oleifera* leaves, *Lannea barteri* bark extract, Growth, *Clarias gariepinus*

Introduction

Fish is a source of indispensable amino acids in the diet of human and animals because of its high quality proteins (Shim *et al.,* 2009). Needs for fish products are increasing on daily basis while wild stock is rapidly decreasing due to over exploitation. A typical fisherman believed that fish in wild is a renewable resource that can never be exhausted from stock. Villa-Cruz *et al.* (2009) reported that sustaining fish supplies from captured fisheries will not meet growing demand for fish product globally rather aquaculture sustainability; which its activity requires high quality feeds which contain necessary nutrients and complementary feed additives to keep aquatic organisms healthy, fast growth, environmental friendly, and disease resistant (Eid and Mohamed, 2008).

The palatability of diets and feeding rate are also of paramount importance to the growth performance of the farmed fish species. Thus, formulation of a diet that meets up with the nutrient requirements of the cultured fish is imperative for the rapid growth rate and survival of aquaculture fish species (Ajiboye *et al.*, 2012). There is large number of feed additives available to improve fish growth performance. Some of these additives are chemical products especially hormones and antibiotics which may have both short and long term unfavourable side effects. The use of antibiotic growth promoters (AGPs) as feed additives in the aquaculture industry has been criticised by government policies and consumers because of possible development of microbial resistance to these products and their potential harmful effects on human's health (Baruah*et al.*, 2008). World Health Organization encourages using of medicinal herbs to substitute or minimize the use of chemotherapy (Levic*etal.*, 2008; Adekunle, 2012). Natural plant products have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation, tonic and immune stimulation and to antimicrobial properties in fin fish and shrimps' larvae culture (Citrasu, 2010). From the forgoing, this study seeks to investigate the effect *Moringa oleifera* leavesand stem bark of *Lannea barteri* and oxytetracycline antibiotic as an additive on the growth performance of *Clarias gariepinus*.

Materials and Methods

Moringa oleifera leaves were obtained from horticultural garden, Department of Crop Production Federal University of Technology, Minna. The leaves were taken to Fisheries laboratory; air dried at room temperature for 5 days and grounded to powdery form (Nweze *et al.*,2014), and sieved. *Lannea bateri* bark was obtained from Gupa-Miffivia, Lapai Local Government Area of Niger State. It was cut into smaller sizes, pounded and sun dried for 2 days, ground into powdery form (Koné *et al.*, 2011), and sieved. They were kept in a safe place until used.

50grams each of the plants were measured and soaked into 500ml of ethanol and distilled water following the procedures of (Nweze and Nwafor, 2014) for 72 hours in 1000ml bottle. The bottles were tightly covered to prevent evaporation and thoroughly shake at intervals. The soaked materials were doubled filtered and then, the concentrated liquid collected were fed to 1 litre flask clipped to a rotary evaporator (RE300) machine rotating on a water bath boiling steadily at 100?C and the solvent were removed. The extracts were poured into 10ml bottle, exposed to air until ethanol solvent finally removed. The extracts were covered and stored at room temperature until used. The same procedure was used for aqueous extract.

Eight different experimental diets were formulated for the experiment. Each of the experimental diet was formulated to contain 45% Crude protein (CP) as compare with the label of the package in the commercial reference diet (CRD) which was used as the control. The proximate analysis of the major ingredients was determined before the diets were formulated while inclusion levels of each ingredient represented in each treatment is shown in Table 1.

The experimental diets were formulated using Pearson square method and compounded into pellet using manual pelletizer. The diets were prepared by individually weighing of each component, and were prepared based on individual treatment formula and thoroughly mixed to ensure homogeneity. The pellets were then made into crumbles so as to make it easier for fish while feeding. The feeds were packed in polythene, labeled and stored in deep freezer at -4°C before the commencement of the experiment.

Five hundred and forty (540) *Clarias gariepinus* fingerlings with an average body mass of 4.60±0.02g were acclimated for two weeks before being randomly distributed into 27 plastic tanks of 50 litres capacity filled with 20 litres volumes. Each of the tanks contains 20 fish, in triplicate representing 9 dietary treatments. Fish in the group of treatment 1 represent control diet while the rest were fed the tested diets. Water flow rate was maintained at 1.5 litres per minute and water quality parameters were measured weekly. Results of all the water quality parameters measured were within international recommended ranges (Table 3). Fish were fed 5% of their body weight per day in two equal meals between 10am and 4pm for 56 days. All the fish in each tank were weighed (with what) fortnightly and feeding rates were adjusted accordingly. Uneaten feeds were siphoned from each tank and kept according to each treatment while faecal matters were also siphoned and kept for faecal analysis. At the end of the experiment (56 days), growth parameters and feed utilization indices were determined using the following formulae:

- Mean Weight Gain (g) = Mean Final Weight (g) Mean Initial Weight (g).
- Specific Growth Rate (%/Day) = (ln W2- ln W1/ T2 T1) × 100.
- Food Conversion Ratio = Weight of Feed (g) / Weight Gain (g) by fish.
- Protein Efficiency Ratio = Live Weight Gain (g) /Crude Protein Fed (g).
- Apparent Net Protein Utilization (%) = Protein in Fish Carcass (g) at the end of the study - Protein in fish Carcass (g) at the end of the study/PI × 100

Evaluations of growth parameters of the diets were subjected to one-way analysis of variance (ANOVA) using $p>0.05$ significance level to test for significant difference. The parameters of mean comparison were applied according to Duncan multiple range test (1955). All the statistics were computerized using stat graphics (version 3.0) and Minitab (version 9.2) packages.

Results and Discussion

Growth parameters and nutrients utilization of experimental fish (*C. gariepinus*) fed supplemented plants additives of three different extraction methods (whole, aqueous and ethanol) in their diets is

shown in Table 2 with significant differences ($p<0.05$). The survival rate of the experimental fish was between 90 to 98%. There was improvement on the growth performance (Table2) of the fish fed plant additives diets even though their performances were significantly lower ($p<0.05$) than those fed of control diet Non-Additive diet (NAD) and Oxytetracycline (Anti-biotic -ANTB). However, among the diets containing plant additives, the best growth response was achieved in Lannea plant whole (LWL) based diet (Table 2). There was no significant difference ($p>0.05$) in the Specific Growth Rate (SGR) of the fish fed CRD, NAD and ANTB as the values were 2.46% ± 0.21, 2.48% ± 0.17 and 2.23% ± 0.23 respectively. There were no significant difference ($p>0.05$) in all values recorded for Feed Conversion Ratio (FCR) while CRD, NAD and ANTB. Similarly, there were no significant differences ($p>0.05$) in the Protein Efficiency Ratio (PER); however, the Apparent Net Protein Utilization (ANPU) exhibited a significantly high ($p<0.05$) value in LEE inclusion diet and lowest value in ANTB diet.

The results of the experiment conducted indicated that, the experimental fishes accepted all the diets. However, the Specific Growth Rate (SGR) obtained from the group of fish fed Commercial Reference Diet (CRD), Non-additive diet (NAD) and Anti-biotic (ANTB) were significantly higher ($p<0.05$) than SGR values for the additive diets. This is in disagreement with the findings of Eid and Mohamed (2008), Khattab *et al.* (2004) and Mohamed *et al.* (*2007*) who reported that, *Oreochromis niloticus* fingerlings fed on diet supplemented by probiotics exhibited greater growth than those fed with control diets. The feed conversion ratio for all the (diets additives and non-additive) were in agreement with that of the values obtained by Nnaji and Okoye (2005); Ovie and Adejayan (2010). The latter reported that, FCR values ranging between 1.10 and 1.23 for *Clarias gariepinus* are not significantly different ($p>0.05$) in the various growth parameters even though they were fed with varying levels of garden snails *Limicolaria* spp. The high survival rate recorded could be as a result of constant monitoring of water quality and phytonutrients content of additives as the highest survival rate was recorded in the group of fishes that were fed plant additives.

Table 1: Diets Formulation and Chemical Compositions of Experimental Diets

Feed Ingredients (g)	Diet 1 (CRD)	Diet 2 (NAD)	Diet 3 (MWL)	Diet 4 (MAE)	Diet 5 (MEE)	Diet 6 (LWL)	Diet 7 (LAE)	Diet 8 (LEE)	Diet 9 (ANTB)
F M (72.4% CP)	-	50.4	49.4	49.4	49.4	49.4	49.4	49.4	49.4
MM (14.0%)	-	44.6	43.6	43.6	43.3	43.6	43.6	43.6	43.6
VMP	-	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Oil	-	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Additive	-	-	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Total	-	100.00	100	100	100	100	100	100	100
Compositions (%)									
Moisture	4.12	2.3	2.48	2.84	2.22	2.55	2.82	2.38	2.41
Crude Protein	46.6	45.18	44.86	44.89	44.10	44.50	44.50	44.75	45.43
Ether Extract	6.00	8.50	8.80	9.70	9.00	8.40	9.90	9.20	9.30
Crude Fibre	2.86	2.30	2.6	2.67	2.58	2.40	2.67	2.58	2.58
Ash	17.40	14.18	18.60	17.01	15.57	17.40	16.80	13.58	14.9
NFE	23.02	27.54	22.66	22.89	26.30	24.75	23.25	27.51	25.38
Total	100	100	100	100	100	100	100	100	100

CRD = Commercial Reference Diet, NAD = Non-Additive Diet, Moringa Whole Diet, MAE = Moringa Aqueous Extract Diet, MEE = Moringa Ethanol Extract Diet, LWL = Lannea Whole Diet, LAE = Lannea Aqueous Extract Diet, LEE = Lannea Ethanol Extract Diet, FM = Fish meal, VMP = Vitamin and Mineral Premix and CP = Crude Protein.

Table 2: Growth parameters and Nutrient Utilization of *Clarias gariepinus* fingerlings Fed Experimental Diets

Growth Parameters	Diet 1 (CRD)	Diet 2 (NAD)	Diet 3 (MWL)	Diet 4 (MAE)	Diet 5 (MEE)	Diet 6 (LWL)	Diet 7 (LAE)	Diet 8 (LEE)	Diet 9 (ANTB)
MIW (g/fish)	4.61^{a}	4.59^{a}	4.63^{a}	4.63^{a}	4.62^{a}	4.55^{a}	4.60^{a}	4.57^{a}	4.55^{a}
MFW (g/fish)	18.37^{a}	18.48^{a}	11.33^{c} 0.54	11.46^{c} 0.99	13.06^{b}	13.63^{b}	11.85^{c}	12.87^{bc}	15.99^{ab} 2.11
MWG (g/fish)	13.76^{a}	13.87^{a}	6.70^{cd}	6.83^{cd}	8.45^{bc}	9.08^{b}	7.26^{c}	8.30^{bc}	11.42^{ab}
SGR (%/day)	2.46^{a}	2.48^{a}	1.60^{b}	1.61^{b}	1.85^{b}	1.96^{b}	1.69^{b}	1.85^{b}	2.23^{a}
FCR	1.10^{a}	1.08^{a}	1.57^{a}	1.57^{a}	1.39^{a}	1.29^{a}	1.51^{a}	1.39^{a}	1.12^{a}
PER	2.04^{a}	2.06^{a}	1.42^{ab}	1.42^{ab} 0.13	1.60^{ab}	1.73^{ab}	1.48^{ab}	1.60^{ab}	2.00^{a}
ANPU (%)	91.40^{ab}	79.06^{bc} 6.64	34.81^{cd} 1.71	66.45^{c} 6.05	82.18^{b}	31.72^{de} 1.41	33.19^{d}	93.28^{a} 94	5.57^{e}
Survival (%)	95.00^{ab}	93.33^{b}	98.33^{a} 2.89	90.00^{c} 8.66	95.00^{ab} 0.00	91.67^{bc} 5.77	98.33^{a}	98.33^{a}	93.33^{b} 7.64

Average values on the same row carrying similar superscripts are not significantly different from each other (P>0.05).
MIW = Mean Initial Weight, MFW = Mean Final Weight, MWG = Mean Weight Gain, SGR = Specific Growth Ratio, FCR = Food Conversion Ratio, PER = Protein Efficiency Ratio, ANPU = Apparent Net Protein Utilization.

Table 3: Ranges of Weekly Average Water Quality Parameters Monitored in all Experimental Tanks During56 Days Feeding Trial

Parameters	Ranges	NIS, 2007
Temperature (°C)	21.8-27.1	-
pH	6.9-8.6	6.5-8.5
Conductivity	116.3-224.0	100-500
Total Alkalinity (mg/l)	78.7-93.3	-
Dissolved Oxygen (mg/l)	3.0-5.7	3-7l

Conclusion

The results from the experiment indicated that, the plants used for the experiments were not toxic to fish health. This is an encouragement for the use of medicinal plants as additive in aquaculture diets. The fishes fed on Lannea Whole (LWL) diet and Lannea Ethanol Extract (LEE) diets expressed better growth compared with Moringa based diets. Further experiments will be required in subjecting the additives to various inclusion levels to establish the optimal inclusion in the fish diet. Achieving this will discourage fish farmers from using antibiotic as growth promoting additive which have been established to impact residual effects on the consumers.

References

Adekunle A. D. (2012). Effect of herbal growth promoter feed additive in fishmeal on the performance of Nile Tilapia (*Oreochromis niloticus* (L)). Egypt. *Acad. J. Biolog. Sci.*,4(1): 111-117.

Ajiboye O. O., Yakubu A. F. and Adams T. E. (2012). A Perspective on the Ingestion and Nutritional Effects of Feed Additives in Farmed Fish Species. *World Journal of marine sciences* 4(1)87-101.

Baurah,K., Norouuzitallab, P., Debnath, D., Pal, A. K. &Sahu, N. P. (2008). Organic acids as non-antibiotic nutraceuticals in fish and prawn feed. *Aquaculture Health International* 12: 4-6.

Citarasu, T. (2010). Herbal biomedicines: A new opportunity to aquaculture industry. Aquacult. Int., 18: 403-404.

Duncan, D. B. (1955) "Multiple Range and Multiple F test". Biometrics 11:1-42.

Eid, Abdel Hamid and Mohamed K.A. (2008). Effect of using probiotic as growth promoters in commercial diets for monosex Nile Tilapia (*Oreochromis nilotucus*) Fingerlings. *8th International Symposiumon Tilapia in aquaculture. Department of animal and fish production, Faculty of Agriculture, Suez Canal University,41522, Ismailia, Egypt.*

Khattab, Y. A., Shalaby, S. M.., Sharaf, H. I. Marakby, E. L. and &Rizhalla, E. H. (2004). The Physiological Changes and Growth Performance of Nile Tilapia (*Oreochromis niloticus*) after Feeding with Biogen® as growth promoter. Egypt J. Aquatic Biol. Fish 8: pp 145-158

Koné,W. M. Soro, D., Dro, B., Yao, K. &Kamanzi, K. (2011). Chemical Composition, Antioxidant, Antimicrobial and Acetylcholinesterase Inhibitory Properties of *Lannea Barteri* (Anacardiaceae). *Australian Journal of Basic and Applied Sciences*, 5(10): 1516- 1523.

Levic J., Sinisa M., Djuragic O. and Slavica S. (2008). Herbs and organic acids as analternative for antibiotic- growth-promoters. *ArchivaZootechnica*11: 5-11.

Mohamed, K. A., Baida, Abdel Fattah & Eid, A. M. S. (2007). Evaluation of Using Some Feed Additives on Growth Performance and Feed Utilization of Monosex Nile Tilapia (*Oreochromis niloticus*). M.sc Thesis, Saba. Basha. Alex University,215pp.

Nnaji, C. J. & and Okoye, F. C. (2005). Effect of Substituting Fish meal with Grasshopper meal in the diet of *Clarias gariepinus* fingerlings In: Aroaye, P. A. (Ed) proceedings of 19th Annual Conference of Fisheries Society of Nigeria. Ilorin: 29th November, -3rd December, FISON.

Nwerze, N. O. and Nwafor, F. I. (2014). Phytochemical, Proximate and Mineral Composition of Leaf Extracts of *Moringa oleifera* Lam. From Nsukka, South-Eastern Nigeria. 91-103.

Ovie, S. O. and Adejayan, A. B. (2010). Effects of Supplementing Fish Meal with Garden Snail (*Limicolaria* Spp.) in *Clariasgariepinus* Diets. Sciencepub 2: 58-62.

Shim, S. M., Ferruzi, M. G., Kim, Y. C., Janle, E. M. &Santerre, C. R. (2009). Impact of Phytochemical-rich foods on Bioaccessbility of Mercury from Fish.*Food chemistry* 112: pp46-50.

Villa-Cruiz, V., Davilla, J. Viana, M. T. and Vaqquez-Duhalt, R. (2009). Effect of broccoli (*Brassica oleracea*) and its phytochemical suforaphane on balanced diets on the detoxification of enzyme levels of Tilapia (*Oreochromis niloticus*) exposed to carcinogenic and mutagenic pollutant. *Chemosphere* 74: pp 1145-1151.