

Growth performance and sensory parameters of African catfish (*Clarias gariepinus*) fed with a sublethal dose of neem leaf extract, and its antibacterial effects

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ABSTRACT

The antibacterial effects of Neem leaf, the growth performance and sensory qualities of African catfish fed with Neem leaf inclusion were investigated in this study. Ninety (90) post-juvenile *Clarias gariepinus* (42.40 ± 2.50 g) were used. Experimental groups in triplicate were exposed bi-weekly to 3.5% and 7% LC₅₀ of Neem leaves aqueous extract infused in commercial floating feed for four weeks, while the control group was exposed to untreated feed. The whole body length and weight of randomly sampled fish were taken for organo-somatic indices. Gills and skin samples were collected for bacteriological screening, while fish muscles were prepared for sensory acceptability tests. Mean feed intake was significantly (P<0.05) influenced by Neem leaf in the three treatment concentrations with an insignificant difference between the treated groups, but it was higher than in the control group. Mean weight gain was significantly higher (P<0.05) in the Neem fed fish than the control but insignificant the differences between treatment doses were insignificant, while the feed conversion ratio was significantly lower (P<0.05) in the Neem fed groups compared to the control, but the difference was insignificant between the treatment doses. All groups were apparently healthy looking with 100% survival. There was a considerable reduction in total bacterial count in both the gills and skin with the increase in Neem concentration. *Micrococci* spp. and *Bacillus subtilis* were not isolated in the gills but were present in the skin, while *E. coli* and *Pseudomonas fluorescens* were not suppressed in the skin. There was no significant difference (P<0.05) in all the sensory parameters across all groups. The inclusion of Neem leaf extract was shown to have a high antibacterial effect coupled with excellent growth performance and general acceptability of fish tissue.

Key words: aquaculture; Neem; antibacterial; growth; *Clarias gariepinus*

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Introduction

Aquaculture is one of the fastest growing food production sectors in the world, with global inland aquaculture production increased from 29.9 million tonnes in 2007 to 41.9 million tonnes in 2012 (FAO, 2014). Nigeria has not been left out of this trend as it provides a suitable and cheap substitute for dietary protein. With this production surge, aquaculture facilities are increasingly relying on the heavy input of formulated feeds, antibiotics, antifungal products, and agrochemicals for increased production. The intensive use of antibiotics, growth boosters and chemicals has raised questions about the impact of veterinary medicines on livestock production, the environment and on human health.

The Neem plant (*Azadirachta indica*) is a non-leguminous multi-purpose tree which belongs to the family *Meliceae*, otherwise known as Nim tree and Margosa tree (ARBONNIER, 2004). It is a common, thriving, fast growing, evergreen and readily available plant in Nigeria, believed to be native to the Indian subcontinent (GIRISH and SHANKARA, 2008) and Senegal (ORWA et al., 2009). It has been reported to boost the immune system of humans, and several animals and fish species (MUKESH et al., 2012; KWAWUKUME et al., 2013; SHAILENDER et al., 2013). HARIKRISHNAN et al., 2003, also reported that the Neem plant has antibacterial, antifungal, antiviral and pesticidal activities, while at the same time improving general growth and health performance, with no toxic effect to vital organs.

Neem, despite its bitter taste, has been introduced and investigated in the composition of livestock diet as Neem Seed Cake, due to the presence of essential amino acids, crude protein, fibre, Sulphur and Nitrogen (ESONU et al., 2005; ESONU et al., 2006; OFORJINDU, 2006; UKO and KAMALU, 2007; OGBUEWU, 2008) and Neem Leaf Meal (UKO and KAMALU, 2001; BAROA et al., 2006; ODUNSI et al., 2009; ARUWAYO and MAIGANDI, 2013), as a replacement for groundnut or soya beans due to its high protein component without any adverse effects. NNENNA and ANIEBO (2013) in their work on broiler chicks demonstrated the potentials of Neem leaf extracts as a source of nutrients needed by animals. Their report presented the comparable weight gain of the birds fed Neem leaf extract to the control, indicating that the quantity of toxic factors such as terpenes and limonoids (OGBUEWU et al., 2011; KABEH and JALINGO, 2007) in the Neem leaf was minimal and did not impair its nutrient availability, digestion, absorption and utilization.

Several researchers have investigated feed consumption and weight gain activity of Neem in animals. GOWDA and SASTRY, (2000) and UKO and KAMALU, 2008 suggested that raw Neem toxicity and the presence of bitter compounds impair feed intake, while experimental removal of the bitter toxicant - triterpenoids from Neem cake with alkali and caustic soda increased Neem feeding value and protein utilization, with

impressive growth in poultry birds (NAGALAKSHMI et al., 1996; OGBUEWU et al., 2011). Studies on Neem in animal production have been mostly focused on its medicinal uses, mostly as an anthelmintics agent (CHANDRAWATHANI et al., 2006; TIWARY and PANDEY, 2010), while most studies in the area of animal nutrition have involved the use of the seeds as a protein source in animal feed (GOWDA and SASTRY, 2000; OGBUEWU et al., 2011).

Neem leaves are high in crude protein, though with wide variations in the reported values, which may be due to varietal differences in the plant. BAIS et al., 2002 and BHOWMIK et al., 2008 reported crude protein concentrations between 17.5% and 18.7%, 9.7% (RAMANA et al., 2000) and 20.9% (OGBUEWU et al., 2011). According to RAMANA et al., 2000, Neem leaves contain 38.0% Neutral Detergent Fibre (NDF) and 27.0% Acid Detergent Fibre (ADF) levels while BHOWMIK et al., (2008) reported 11.3%. Its calcium content ranges between 1.48% and 1.53%, which is related to the calcium content (1.51%) available in Sesbania leaves, reported by NGAMSAENG et al., 2006. Neem leaves have been reported to be deficient in copper and manganese (NIRANJAN et al., 2008), zinc and phosphorus (RAO et al., 2011). PAENKOU (2010) reported that Neem leaves can replace up to 50% of soya bean meal in ruminant diets with no negative effects on feed intake, dry matter and fibre digestibility, as well as body weight gain. CHANDRAWATHANI et al., 2006 and TIWARY and PANDEY, 2010, revealed that there are also many scientific reports on the anthelmintic properties of Neem leaves and extracts in ruminants, which include *Haemonchus contortus* sensitivity in fed animals. Improved performance with Neem leaves has also been reported for poultry (SONAIYA, 1993). This study was therefore aimed at ascertaining the antibacterial and growth promoting properties of Neem leaf extract in cultured fish feed.

Materials and methods

Sample source and aqueous neem leaf preparation. A total of ninety (90), ten-week-old African Catfish (*Clarias gariepinus*) (post juveniles) (42.40 ± 2.50 g) were acquired. They were acclimatized and subsequently transferred to experimental fibre plastic tanks, as described by ONIOVOSA et al., 2017. The juveniles were stocked at a density of 10 fish per 50 litre water tank. An aqueous extraction of Neem leaves was prepared according to the method described by CROSS et al. (2004) while incorporation of the aqueous extract into the fish feed at 3.5% and 7% concentrations, and the experimental set up were done as described by ONIOVOSA et al., 2017.

Fish were randomly sampled from each experimental group at the start of the experiment with measurement of their weight and length. At the end of the experiment, the length and whole body weight of randomly sampled fish, as well as the weight of the various organs were recorded for organo-somatic indices. Gill and skin samples

were collected for bacteriological screening, while other fish samples were prepared for sensory acceptability tests.

Fish samples for sensory acceptability test were washed with clean water, properly labelled and stored on ice in an insulated flask for transfer to the kitchen. They were de-gutted, cut into uniform sizes, washed with clean water and steamed separately with a pinch of iodized salt in 30 cL of clean water for 10 minutes and allowed to cool to a reasonable serving temperature. A similar part of each fish was presented for evaluation to each member of the panel (Five (5) semi-trained persons from the university community) in coded digital labels to conceal every information about the samples, in order to avoid bias in judgement. After each sample, mouth rinsing with clean water and a waiting time of about 5 minutes was encouraged for clarity of taste. Palatability and acceptability was subjected to the Quality Index Method (QIM) of sensory quality evaluation.

The sensory parameters were scored by the analysts using the following parameters: Appearance/ coloration (Fish white, dull white, slightly yellowish, yellowish discolouration); Taste /flavour (Sweet, off flavor, slightly bitter, very bitter); Odour (Fresh, neutral, offensive); Overall acceptability (highly acceptable, acceptable, fairly acceptable, not acceptable).

Growth rate evaluation. At the end of the experiment, the Initial Mean Weight (IMW), Final Mean Weight (FMW), Mean Weight Gain (MWG), Percentage Weight Gain (PWG), Mean Feed Intake (MFI), Feed Conversion Rate (FCR) and Survival Rate were calculated and analysed statistically using SPSS, (1999).

$$\text{Mean Weight Gain (MWG)} = W_f - W_i$$

where

W_i means Initial weight (g) and W_f means Final weight (g)

$$\text{Feed Conversion Ratio (FCR)} = \text{Mass of food consumed dry} \times 100$$

$$\text{Increase in mass of animal produced wet} = (\text{Total dry feed fed}) / (\text{Wet weight gain})$$

$$\text{Survival rate (SR)} = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish}} \times 100$$

Table 1. Sensory palatability score form for cooked fish

Quality parameter	Score guide	Score
Appearance/ coloration	White	4
	Dull	3
	Light yellow	2
	Deep yellow	1
Taste /flavor	Sweet	4
	Off flavor	3
	Slightly bitter,	2
	Very bitter	1
Odor	Fresh (sea weed)	3
	Neutral (odor-less)	2
	Offensive	1
Overall acceptability	Highly acceptable	4
	Acceptable	3
	Fairly acceptable	2
	Not acceptable	1
Score total		

Total scores were analyzed for significant difference

Statistical analysis. The values obtained were expressed as mean \pm SEM (standard error of mean). The homogeneity of data was analyzed by one-way analysis of variance (ANOVA) and the Bonferroni's Multiple Comparison Test was used as test for comparison between means using Graph-Pad Prism (version 4.00 for Windows, Graph-Pad Software, San Diego, California, USA). The significances of the P values <0.05 were also considered.

Results

The growth performance of the fish (Table 2) showed that mean feed intake was significantly ($P<0.05$) influenced by Neem leaf in the treatment concentrations (T2 and T3) compared with the control (T1), although there was no significant difference within the treated groups. Mean weight gain was significantly higher ($P<0.05$) in the Neem fed fish compared to the control, but the difference was not significant between the treatment doses. Similarly the feed conversion ratio was significantly lower ($P<0.05$) in the Neem fed groups compared to the control but there were no significant differences between the treatment doses. All groups were apparently healthy looking with 100% survival.

Table 2. Effect of aqueous Neem leaf extract on fish growth parameters, feed intake, feed conversion ratio and survival rate

Parameters	T1 (0%)	T2 (3.5%)	T3 (7%)
Initial Mean Weight (g)	39.58 ± 2.10 ^a	42.45 ± 2.54 ^a	40.92 ± 1.98 ^a
Final Mean Weight (g)	67.57 ± 2.46 ^b	77.64 ± 4.44 ^a	76.75 ± 4.00 ^a
Mean Weight Gained (g)	27.99 ± 0.73 ^b	35.18 ± 1.93 ^a	35.84 ± 2.02 ^a
Percentage Weight Gain	70.83 ± 3.61 ^a	82.90 ± 1.21 ^b	87.56 ± 0.72 ^c
Mean Feed Intake (g/day)	26.24 ± 0.93 ^b	29.92 ± 1.56 ^a	29.43 ± 0.82 ^a
Feed Conversion Ratio	0.94 ± 0.01 ^a	0.85 ± 0.00 ^b	0.82 ± 0.03 ^b
Survival Rate	100 ± 00 ^a	100 ± 0.00 ^a	100 ± 0.00 ^a

Means with the same superscripts are not significantly different (P < 0.05).

Antibacteriological effects. The bacterial organisms isolated from the experimental fish samples were; *Escherichia coli*, *Micrococci* spp. *Pseudomonas fluorescens*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Bacillus megaterus*, *Proteus* spp. and *Klebsiella*.

Table 3 showed the considerable reduction in total bacterial count in both the gills and skin of sampled fish with an increase in Neem concentration. *Micrococci* spp. and *Bacillus subtilis* were not isolated in the gills (Table 4) but were present in the skin (Table 5). *Escherichia coli*, *Bacillus megaterus* and *Proteus* spp. in the gills were resistant to the Neem leaf extract at both concentrations (Table 4), while *Aeromonas hydrophila* was only susceptible at the 7% (T3) concentration in the gill. In the skin sample, *E. coli* and *Pseudomonas fluorescens* were resistant to Neem leaf extract at both concentrations, *Micrococci* spp. *Klebsiella* spp. and *Bacillus megaterus* were susceptible to Neem leaf extract at both concentrations while *Aeromonas hydrophila*, *Bacillus subtilis* and *Proteus* were only susceptible at the 7% (T3) concentration.

Table 3. Total bacterial count × 10⁶ cfu/mL in the gills and skin of sampled fish at different neem concentrations

Fish organ	T1 (Control)	T2 (3.5%)	T3 (7%)
Gill	5.8	5.2	4.0
Skin	6.1	3.6	3.3

Table 4. Bacterial organisms isolated from fish gills

Gill	<i>E. coli</i>	<i>Micrococci</i> spp.	<i>Pseudomonas fluorescens</i>	<i>Aeromonas hydrophila</i>	<i>Bacillus subtilis</i>	<i>Proteus</i> spp.	<i>Klebsiella</i> spp.	<i>Bacillus megaterus</i>
T1	+	-	+	+	-	+	+	+
T2	+	-	-	+	-	+	-	+
T3	+	-	-	-	-	+	-	+

Table 5. Microbial organisms isolated from fish skin

Skin	<i>E. coli</i>	<i>Micrococci</i> spp.	<i>Pseudomonas fluorescens</i>	<i>Aeromonas hydrophila</i>	<i>Bacillus subtilis</i>	<i>Proteus</i> spp.	<i>Klebsiella</i> spp.	<i>Bacillus megaterus</i>
T1	+	+	+	+	+	+	+	+
T2	+	-	+	+	+	+	-	-
T3	+	-	+	-	-	-	-	-

Sensory score result analysis. The sensory scores results (Fig. 1) showed no significant differences ($P < 0.05$) in the color, taste, odor and general acceptability across all groups, although a slight coloration occurred at 3.5% concentration on visual observation.

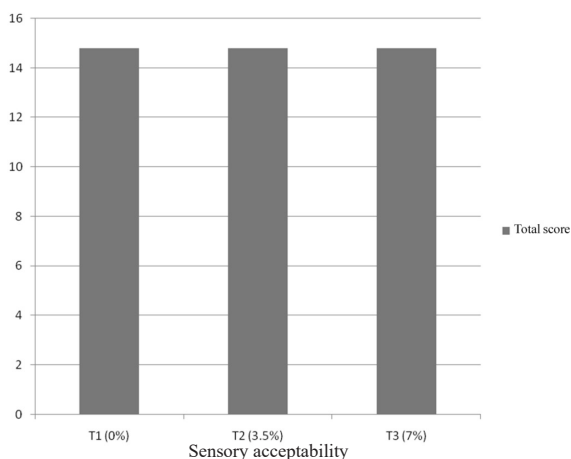


Fig. 1. Pictorial presentation of sensory scores. Sensory acceptance graded at the same level for all concentrations of Neem used in this experiment.

Discussion

Growth performance. The average feed intake of fish was not affected by the bitter taste of the Neem leaf, contrary to the findings of GOWDA and SASTRY (2000), making the process of detoxifying Neem for the purpose of increased palatability and acceptance in feed, as proposed by NAGALAKSHMI et al. (1996), not necessary for catfish. This feed intake and weight gain report is in agreement with the work of OGBUEWU et al. (2011) and KABEH and JALINGO (2007) which stated that in birds, the quantity of toxic factors, such as terpenes and limonoids in Neem leaf did not impair the nutrient availability, digestion, absorption and utilization of feed. Contrary to the report of BONSU et al., (2012) on broilers fed with Neem leaf, the result obtained in this research showed the positive significant effect of Neem leaf inclusion on both weight gain and feed conversion efficiency of juveniles of *Clarias gariepinus*, which is in agreement with the report by TALPUR and IKHWANUDDIN (2012) for *Lates calcarifer* fingerlings.

Bacteriological response. The antibacterial property of Neem leaves in the gills and skin of *Clarias gariepinus* was shown in the decrease in the total bacteria count in response to an increase in Neem leaf treatment concentrations, which is in agreement with reports by several researchers (PEDGE and AHIRRAO, 2012; RAVIKUMAR et al., 2011; MADHURI et al., 2012). The effect of Neem on *Aeromonas hydrophila* in this study is in line with the work of HARIKRISHNAN et al., (2003) on carp fish, as the 7% concentration inclusion of Neem leaf extract successfully suppressed *Aeromonas hydrophila* in all samples. The suppressed bacterial pathogens in this study are also in agreement with the report by DHAYANITHI et al. (2010) on ornamental marine fishes. *Klebsiella*, *Bacillus subtilis*, *Micrococci* spp. were successfully suppressed by the inclusion of Neem in the fish diet, similar to the report by RAM et al., (2002) on *Klebsiella pneumoniae*, but contrary to this same author on *E. coli* as all experimental groups, including the 7% treatment concentration of Neem remained positive to *E. coli*. *Pseudomonas fluorescens* was also not affected at these concentrations of Neem leaf extract.

Sensory quality. Although, CLAUSEN et al., (1985) discouraged the use of products with an unpleasant taste in animal feed, saying that there is a chance that the unpleasant bitter taste of the plant might have impact on the smell and taste of the meat, the results of this research demonstrate the opposite as samples of fish fed Neem leaf in their diet presented no trace of the taste of the Neem in terms of coloration, odor or taste, even at the 7% concentration. As observed regarding the sensory acceptability of *Clarias gariepinus* in this present study, there was no significant difference between the treatment and the control group, which was similar to the report by BONSU et al., (2012), who recorded a mild bitter taste change in the meat of broiler chicken fed a relatively high percentage of Neem leaf, although it had positive sensory acceptability.

Conclusion

Neem plants are readily available, low in toxicity, environmentally friendly and rich in several biologically active components that give them multifunctional ability. As revealed in this experimental research, aqueous Neem leaf extract infused into catfish feed at sub-lethal concentrations has no negative effect on the sensory quality values and general acceptability of the fish muscle. Neem leaf inclusion in feed has a remarkable positive effect in the suppression of bacteria growth, as well improved general growth performance (weight gain and feed conversion efficiency). Due to the growth- promoting and antibacterial properties of Neem leaf extracts, funding of research leading towards its standardization in the management of aquatic animal diseases, as well as livestock health, should be encouraged.

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UBIOGORO, O. E., S. A. ALARAPE, A. B. SAKA, O. K. ADEYEMO: Rast i senzorni pokazatelji afričkog soma (*Clarias gariepinus*) hranjenog subletalnom dozom ekstrakta lišća nima (azadirachte) i njegovi antibakterijski učinci. *Vet. arhiv* 89, 709-721, 2019.

SAŽETAK

U ovom su radu istraženi antibakterijski učinci lišća nima, kao i rast i senzorni pokazatelji afričkog soma (*Clarias gariepinus*) hranjenog ovim lišćem. Upotrijebljeno je 90 odraslih afričkih somova ($42,40 \pm 2,50$ g). Pokusna je skupina izložena tijekom dva tjedna 3,5 % i 7 % LC50 vodenom ekstraktu lišća nima dodanog u komercijalnu hranu tijekom četiri tjedna, dok je kontrolna skupina hranjena netretiranom hranom. Određena je tjelesna dužina i masa nasumično odabranih riba za provjeru organoleptičkih pokazatelja. Škrge i uzorci

kože prikupljeni su za bakteriološku analizu, dok su uzorci mišića pripremljeni za senzornu analizu. Prosječna konzumacija hrane bila je pod znakovitim utjecajem ($P < 0,05$) lišća nima, neovisno o koncentraciji, u odnosu na kontrolnu skupinu. Prosječan prirast bio je znakovito viši ($P < 0,05$) u riba hranjenih liščem nima u odnosu na kontrolnu skupinu, dok je konverzija hrane bila znakovito niža ($P < 0,05$) u skupini hranjenoj liščem nima, neovisno o dozi. Riba u svim skupinama bile su naizgled zdrave uz 100%-tno preživljenje. Utvrđen je zamjetan pad brojnosti ukupnih bakterija u škrgama i na koži s porastom koncentracije lišća nima. Bakterije *Micrococci* spp. i *Bacillus subtilis* nisu izolirane iz škrge, ali su utvrđene u koži, kao i *E. coli* i *Pseudomonas fluorescens*. Nisu utvrđene znakovite razlike senzornih pokazatelja. Dodatak lišća nima ima visok antibakterijski učinak praćen odličnim pokazateljima rasta te općom prihvatljivošću tkiva ribe za konzumaciju.

Cljučne riječi: akvakultura; nim; antibakterijski; rast; *Clarias gariepinus*
