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Effects of *Alstonia boonei, Spondias mombin* and *Morinda lucida* leaves extracts on growth and haematology of African Catfish (*Clarias gariepinus)* Juveniles

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Abstract

This study evaluated the growth performance and haematology of *C. gariepinus* fed *A. boonei*, *S. mombin* and *M. lucida* leaves extracts for 49 days. A total of 168 fingerlings were randomly allotted to seven treatment of twenty-four fish each at triplicate of eight fish per tank. The seven treatments are diet 1 (control, 0 mL), diet 2 (2mL *A. boonei*), diet 3 (4mL *A. boonei*), diet 4 (2mL *S. mombin*), diet 5 (4mL *S. mombin*), diet 6 (2mL *M. lucida*) and diet 7 (4mL *M. lucida*). Diets had no effects ($p > 0.05$) on mean weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio across treatments. However, fish fed diet 3 (4mL *A. boonei*) recorded the highest values for mean weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and the best value (1.22±0.23) for feed conversion ratio when compared with others. With the exception of MCV and MCHC, no significant differences ($p>0.05$) were recorded in the values of haematological indices across treatments. The results of this study showed that 4 mL *A. boonei* could be used to supplement the diet of *C. gariepinus* for optimal growth and improved health.

Keywords: Herbal extracts**,** Growth performance, Haematology, Catfish

Introduction

The main concern in fish production is to ensure good performance and quality products of farmed fish. This is limited by poor quality fish seed and the prevalence of diseases in farmed fishes which are major constraints to sustainable aquaculture. In this respect, the common practice in growth promotion, prevention and treatment of diseases in aquaculture include the use of chemotherapeutic agents such as antibiotics. However, using antibiotics for a long time on fish can induce nephrotoxicity and liver damage (Hentschel *et al.*, 2005), may lead to the presence of residual antibiotics in fish tissue and fish products (Samanidou and Evaggelopoulou, 2007), may lead to

potential development of antibiotic resistant bacteria, environmental pollution and other adverse effects on fish population (Ringø *et al.,* 2004). In view of the above reasons, many countries have forbidden the use of certain chemotherapeutics and also refuse to import aquaculture products treated with antibiotics and chemicals (Yousefian and Mousavi, 2011).

Therefore, the desire for eco-friendly as well as cost effective feed additives in addition to the global demand for safe feed that will promote the growth of fish, seed quality and fish health, has inspired the search for natural additives such as plant extracts to be used in aquaculture feeds.

Plants are useful in the treatment of various diseases (Olowokudejo *et al.*, 2008; Kadiri *et al*., 2013) and have been used as traditional medicine since time immemorial to control bacterial, viral and fungal diseases. Medicinal plants are effective alternative agents to treat infectious diseases and can also mitigate many of the side effects that are associated with synthetic antibiotics (Punitha *et al*., 2008). In addition, medicinal plants provide a cheaper source of treatment and greater accuracy than chemotherapeutic agents in this field (Punitha *et al*., 2008).

Practically, all medicinal herbs have active ingredients, comprising of a wide variety of secondary metabolites of phytochemical constituents such as tannins, alkaloids, glycosides, flavonoids, (Pandey and Madhuri, 2010) which are responsible for various biological activities and are significant sources of synthetic and herbal drugs. The herbs/ herbal extracts are used not only against diseases but also as anti-bacteria, growth promoters, appetite stimulants, anti-stress and immune enhancement (Citarasu *et al*., 2002, 2006; Pandey *et al.*, 2012; Sabry *et al*., 2014). According to Pavaraj *et al*. (2011) and Dienye and Olumuji (2014), they reported that medicinal plants (*O. sanctum and Moringa oleifera* leaves meal) induced weight gain in Carp and African catfish respectively due to their therapeutic effect on bacterial infections. According to Kumar *et al*. (2014), other possible mechanisms of action of herbs in the animal for growth promotion and health improvement include changes in the intestinal microbiota, increased digestibility and nutrient absorption, and enhanced nitrogen absorption, improvement of the immune response, morphological and histological modifications of the gastrointestinal tract and antioxidant activity. In addition, Hashemi and Davoodi (2011), confirmed the suitability of herbs as a new class of growth promoters that provide an alternative feeding strategy to replace antibiotic growth promoters.

Plant parts such as leaves, stem bark and roots have bioactive properties of various degrees or concentrations depending on the extraction solvent (Frankic *et al*., 2009) and invariably reflect in their therapeutic efficacy (Akharaiyi and Boboye, 2010). Such plants include Stoolwood (*Alstonia boonei*), Hogplum (*Spondias mombin*) and Brimstone (*Morinda lucida*) with Nigerian Yoruba local names; Ahun, Oruwo and Iyeye respectively (Abo *et al*., 2008).

The aim of this study was to investigate the additive inclusion effects of *A. boonei, M. lucida* and *S. mombin* leaves extracts as possible herbal growth promoters and immune boosters in *Clarias gariepinus* juveniles.

Materials and Methods

Collection and extraction from *Alstonia boonei*, *Spondias mombin* and *Morinda lucida* leaves: The dried leaves of *Alstonia boonei*, *Spondias mombin* and *Morinda lucida* were procured from the local market Oyingbo, Ebute-Metta, Lagos, Nigeria and identified at the Department of Botany, University of Lagos, Nigeria.

Twenty grams (20g) of each dried plant samples was put in separate 250mL conical flasks containing 200mL of ethanol (95%). The mixture was kept for 24hrs on an orbital shaker. After 24hrs, the extract of each plant was filtered using muslin cloth. The extraction was repeated twice after which the filtrates obtained from the extractions were put together and centrifuge at 10,000rpm for 5mins and the supernatant was collected and concentrated using water bath at temperature of 70°C. A greasy final material (crude ethanol extract) was obtained and transferred into screw-cap bottles, weighed, labeled and refrigerated at 4°C for future use (Ojekale *et al.*, 2006).

Experimental Diets: The purified feed ingredients (casein, gelatin, corn starch, cellulose powder, vitamin premix, plant oil and cod liver oil) were mixed manually using hot water to ensure proper gelatinization (Aderolu and Sahu, 2015; Aderolu *et al.*, 2017; Setufe *et al*., 2018). The feed was autoclaved at 121^0C for 15 minutes and pelletized using a filter basket of mesh size 2mm.

The pelletized diets were air-dried indoors for 3 days and stored in air-tight polythene bags prior to use. Seven diets were formulated; diet 1 (control, 0mL extract), diets 2 and 3 (2 and 4mL *A. boonei*), diets 4 and 5 (2 and 4mL *S. mombin*), diets 6 and 7 (2 and 4mL *M. lucida*) (Table 1).

TABLE 1**:** Ingredients composition of experimental diets containing extracts from *Alstonia boonei*, *Spondias mombin* and *Morinda lucida* leaves.

	Control	*A. boonei*		*S. mombin*		*M. lucida*
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet7
Casein	36.98	36.98	36.98	36.98	36.98	36.98	36.98
Gelatin	8.10	8.10	8.10	8.10	8.10	8.10	8.10
Starch	36.47	36.47	36.47	36.47	36.47	36.47	36.47
Cellulose	7.59	7.59	7.59	7.59	7.59	7.59	7.59
Plant oil	6.07	6.07	6.07	6.07	6.07	6.07	6.07
Animal oil	2.02	2.02	2.02	2.02	2.02	2.02	2.02
Vitamin premix	2.73	2.73	2.73	2.73	2.73	2.73	2.73
A. boonei extract	-	2 mL	4 mL	-	-	-	-
S. mombin extract	-	-	-	2mL	4mL	-	-
M. lucida extract	-	-	-	-	-	2mL	4mL
Total	100	100	100	100	100	100	100
Crude protein	39.20	39,20	39.20	39.20	39.20	39.20	39.20

Experimental stock and procedures: Three hundred and fifty *Clarias gariepinus* fingerlings were purchased from U-farms at Ikorodu, Lagos and transported to the nutrition unit of the Department of Marine Sciences, University of Lagos, Nigeria. A 49 day experiment was set up in triplicate per treatment with one hundred and sixty-eight (168) fingerlings (weight range, 5.54-5.57g) were used for the experiment at eight fish per experimental tank

The (52.5 X 33.5 X 21.0 cm^3) tank lids were well perforated to enable adequate ventilation. They were allowed to acclimatize for two weeks during which the fish were fed with 1.2mm coppens feed. The water was changed every two days with water from a borehole to maintain good water quality. The dissolved oxygen ranged from 4.5 to 6.0mg/L while pH ranged from 6.5 to 7.0 during the experimental period.

After the acclimatization, the fish were randomly distributed into the experimental tanks based on the average body weight of 5.55±0.03 g using a CAMRY electronic scale (EK5055, max. 5 kg/11lb, d = 1g/0.05oz).

The fish were hand-fed 5% of their body weight twice daily (9.00 am and 4.00 pm) for 7 weeks with the formulated experimental feed (Table 1). The weight of the experimental fish was recorded at the beginning of the experiment and at the end of every week to determine the average weight gain by bulk-weighing while the quantity of the feed fed for each week was also recorded.

Growth and nutrients utilization parameters measured: At the end of rearing period, the growth and nutrients utilization parameters were estimated for each treatment using the following formulae:

i) Mean weight gain (MWGg) = Mean final body weight (g) - Mean initial body weight (g)

ii) Average feed intake = Feed consumed weekly per tank/No. of fish per tank

iii) Specific Growth Rate (SGR) %/day = $((\ln W_2 - \ln W_1)/\text{Number of days}) * 100$, where, In = Natural logarithm, W_1= initial weight, W_2 = final weight

iv) Feed Conversion Ratio (FCR) = Feed consumed in dry mass (g)/mean weight gain (g)

v) Protein Efficiency Ratio (PER) = Mean weight gain/protein intake

vi) Protein intake = Total feed intake*percentage protein content of feed

Blood sampling for haematological analysis of *C. gariepinus* juveniles: Haematological analysis was carried out on the experimental fish at the end of the experiment. Blood from fish was collected via caudal puncture with the help of disposable 2mL hypodermic syringes and needles (Kori-Siakpere *et al.,* 2005). The blood samples were dispensed into 5

mL bottles containing Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant.

The samples were analyzed at the Department of Haematology Laboratory, University of Lagos (LUTH), Idi-Araba, Lagos. The haematological parameters determined include; Red blood cell, White blood cell, Haematocrit, Haemoglobin, Platelet, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) using semi-automated haematology analyzer (manufactured by Mindray, China).

Statistical analysis: All data collected were subjected to analysis of variance (ANOVA) and Duncan (1955) Multiple Range Tests was used to determine the mean difference among diets at 5% significant confidence level with the aid of SPSS 20.0.

Results

There were no significant differences ($p > 0.05$) recorded among treatments when compared with the control diet in the mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) (Table 2).

However, fish fed 4mL *A. boonei* (diet 3) had the highest values of MWG (4.41±0.66g), SGR (1.17±0.14%/day) and PER (30.62±4.86), while fish fed 2mL *M. lucida* (diet 6) recorded the lowest values for MWG (1.66±0.59g), SGR (0.53±0.17) and PER (11.48±3.67). Also, fish fed diet 3 had the best value for FCR (1.22±0.23) and fish fed diet 6 recorded the worst value for FCR (4.03±1.60). In addition, there was no significant ($p > 0.05$) difference in the values of average feed intake (AFI) in the treated diets compared to the control diet (Table 2).

TABLE 2**:** Growth and nutrient utilization of *C. gariepinus* fed graded levels of *Alstonia boonei, Spondias mombin* and *Morinda lucida* leaves extracts.

Parameter	Control	*A. boonei*		*S. mombin*		*M. lucida*	Significance
	Diet 1(0mL)	Diet 2 (2mL)	Diet 3 (4mL)	Diet 4 (2mL)	Diet 5 (4mL)	Diet 6 (2mL)	Diet7 (4mL)	
FNW (g)	8.07 ± 0.49^{ab}	8.43 ± 1.04^{ab}	10.08 ± 0.62^{b}	8.41 ± 0.45^{ab}	7.44 ± 0.47^{a}	7.16 ± 0.56^{a}	9.45 ± 1.13^{ab}	0.13
INW (g)	5.55 ± 0.00	5.54 ± 0.04^{a}	5.57 ± 0.04	5.40 ± 0.07	5.57 ± 0.04	5.54 ± 0.07	5.56 ± 0.07	0.011
MWG (g)	2.44 ± 0.49 ^{ab}	3.09 ± 1.00^{ab}	4.41 ± 0.66^{b}	2.91 ± 0.38 ^{ab}	1.86 ± 0.51^{a}	1.66 ± 0.59^{a}	3.82 ± 1.20 ^{ab}	0.168
AFI (g)	0.11±0.00^{b}	0.12±0.00^{b}	0.12±0.10^{b}	0.12±0.01^{b}	0.12±0.00^{b}	0.10±0.01^{a}	0.12±0.00^{b}	0.029
SGR (%/day)	0.73 ± 0.12^{ab}	0.90 ± 0.25^{ab}	1.17 ± 0.14^{b}	0.86 ± 0.08^{ab}	0.58 ± 0.14^{ab}	0.53 ± 0.17^{a}	1.03 ± 0.27^{ab}	0.205
FCR	2.36 ± 0.42	2.61 ± 1.10	1.22 ± 0.23	2.04 ± 0.31	3.86 ± 1.44	4.03 ± 1.60	1.89 ± 0.65	0.392
PER	15.93 ± 3.00^{ab}	18.12 ± 5.73^{ab}	30.62 ± 4.86^{b}	17.92 ± 2.37^{ab}	11.48 ± 3.67^{a}	11.37 ± 3.69^{a}	23.11 ± 6.82^{ab}	0.096

*Means in the rows with different superscripts indicate significant differences (P<0.05).

Keys: FNW – final weight, INW – initial weight, MWG – mean weight gain, AFI – average feed intake, SGR – specific growth rate, FCR – feed conversion ratio, PER – protein efficiency ratio.

The results of haematological parameters of *C. gariepinus* juveniles fed graded levels of leaves extracts from three herbs (*A. boonei*, *S. mombin* and *M. lucida*) are shown in Table 3. There were no Significant differences ($p>0.05$) recorded in red blood cell (RBC) count, haematocrit (HTC), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), platelet (PLT) and white blood cells (WBC) count. However, significant differences ($p < 0.05$) were recorded in the values of mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) when control diet was compared with other diets (Table 3). The control group recorded the highest values for RBC (2.74±0.10), HTC (46.50±1.80) and Hb (16.00±0.30) while the lowest values for these parameters (1.96±0.03, 30.70±5.20 and 11.75±0.35 respectively) were recorded for diet 2.

TABLE 3: Haematological parameters of *C. gariepinus* fed graded levels of *Alstonia boonei*, *Spondias mombin* and *Morinda lucida* leaves extracts.

Haematological Parameters	Control	*A. boonei*		*S. mombin*		*M. lucida*	Significance
	Diet 1(0mL)	Diet 2 (2mL)	Diet 3 (4mL)	Diet 4 (2mL)	Diet 5 (4mL)	Diet 6 (2mL)	Diet7 (4mL)	
RBC (10^{12}/l)	2.74 ± 0.10	1.96 ± 0.03	2.17 ± 0.08	2.48 ± 0.25	2.41 ± 0.27	2.03 ± 0.04	2.19 ± 0.21	0.061
HCT (%)	46.50 ± 1.80	30.70 ± 5.20	32.75 ± 2.86	41.05 ± 3.26	39.05 ± 3.83	38.65 ± 0.26	32.15 ± 1.12	0.006
MCV (fl)	179.19 ±3.47^{ab}	163.70 ± 4.09^{b}	149.80 ± 7.04^{a}	166. 40 ± 4.04^{b}	162.05 ± 2.10^{b}	190.60 ± 4.10^{c}	140.25 ± 0.20^{a}	0.000
Hb (g/dl)	16.00 ± 0.30	11.75 ± 0.35	12.60 ± 0.40	14.65 ± 1.52	14.15 ± 1.47	12.20 ± 0.05	12.65 ± 0.89	0.043
MCH (pg)	58.45 ± 0.49	60.10 ± 0.51	58.30 ± 0.51	59.00 ± 0.17	58.70 ± 0.40	60.35 ± 2.35	58.25 ± 1.70	0.514
MCHC (g/dl)	34.45 ± 0.37^{ab}	36.75 ± 0.61^{bc}	39.20 ± 2.19^{cd}	35.50 ± 0.98^{b}	36.20 ± 0.23^{bc}	31.65 ± 031^{a}	41.50 ± 1.15^{d}	0.000
PLT (10^{9}/l)	11.00 ± 0.57	15.50 ± 1.44	14.50 ± 2.59	15.50 ± 0.28	11.50 ± 6.63	15.00 ± 3.46	14.00 ± 4.04	0.007
WBC (10^{9}/l)	9.35 ± 0.75	6.10 ± 0.51	7.70 ± 0.92	10.05 ± 4.55	10.15 ± 2.39	6.90 ± 1.90	6.75 ± 1.75	0.351

*Mean in the rows with different superscripts are significantly different ($p<0.05$).

Keys: RBC-red blood cell, HTC-haematocrit, MCV-mean corpuscular volume, Hb- haemoglobin, MCH-mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, PLT-platelet, WBC-white blood cell.

Discussion

The results in the present experiment are similar to the findings of Tiamiyu (2018), when he fed graded levels of *Talinum triangulare* as an additive to the feed of catfish and recorded weight gain and other nutrient performance parameters not significantly different from the control diet. Equally, Iweala and Obidoa (2009) recorded no change in weight gain in experimental rat fed *Gongronema latifolium.*

Average feed intake equally not significantly different ($p > 0.05$) from fish fed the control diet, though slightly higher in most experimental diets but researchers have attributed drop in average feed intake to palatability related issues (Rodriguez *et al.,* 1996; Tiamiyu, 2018). However, in the present study feed intake was not actually affected at the level being tested. Equally of note is the fact that the inclusion of the plant additive in this study did not result in negative growth as shown in the values obtained for final weights relative to the initial weights and in particular to fish fed 4mL *A. boonei*.

Elsewhere, Frankič *et al.* (2009); Karpagam and Krishnaveni (2014); Kumar *et al.* (2014) and Garg *et al*. (2019), recorded increased growth when they fed some herbal supplemented diets to different fish species. They attributed the performances to improve nutrients utilization, high protein synthesis and absorption, as well as enhanced immune response. Also, there were morphological and histological modifications of the gastrointestinal tract which prevented toxin absorption from the intestine, thereby selectively inhibited the harmful coliforms (Anjaneyulu *et al.,* 1993; Choi *et al*., 2009).

Medicinal plants, which include *Alstonia boonei*, *Spondias mombin* and *Morinda lucida* are rich in secondary metabolites which include volatile oils, saponins, phenolic compounds, tannins, alkaloids, polypeptides and polysaccharides (Tajodini *et al*., 2014), which are the main sources of natural antioxidant and antimicrobial compounds that could aid growth, enhance appetite, stimulate secretion of bile and digestive enzyme activity among others (Asimi and Sahu, 2013).

Haematological characteristics help fish biologists to interpret physiological responses by fish and deviation from normal response may indicate a disturbance in the physiological process (Dienye and Olumuji, 2014). The results of haematological parameters recorded during this study were all within normal ranges for African mud catfish previously quoted by researchers; for red blood cells, Gabriel *et al*. (2007) recoded 2.3-2.9 g/dl, Adeyemo (2007) reported 1.5 g/dl while Agbabiaka *et al*. (2013) reported 1.5-2.15 g/dl. Also, for haematocrit; Musa and Omoreigie (1999) reported 27.58 - 35.50% and

Agbabiaka *et al.* (2013) documented 38 - 44%. Furthermore, for haemoglobin; Osigwe *et al.* (2005) reported 11.3 - 13.4 g/dl, Adeyemo (2007) documented 10.62 g/dl and Agbabiaka *et al.* (2013) recorded 11.75 -16.15 g/dl.

The relatively similar RBC count values across treatments when compared with the control group may be due to better cellular immunity as reported by Ojha *et al*. (2014) and Aderolu *et al.* (2017). This was also validated by Sahu *et al*. (2007) who reported that increased RBC count in *Labeo rohita* fingerlings fed with *Mangifera indica* was an indication of enhanced cellular immunity.

Although, no significant difference ($p>0.05$) was recorded in the values of WBC count across treatments, there were reductions in the values of white blood cell count for diets 2 (2mL *A. boonei*), 3 (4mL *A. boonei*), 6 (2mL *M. lucida*) and 7 (4mL *M. lucida*) compared with the control diet. Nevertheless, the values of WBC count recorded in this study were relatively similar to the results (8.64 - 9.81x10^9/l) reported by Agbabiaka *et al*. (2013) when they fed graded levels of Tigernut based diet to *C. gariepinus.*

Additionally, the values of MCV and MCH observed in the present study were within the range (140 -190fl and 58.25 - 60.35 pg respectively) reported by Osuigwe *et al*. (2005) when they fed catfish with graded levels of jack bean seed meal.

According to Ahilan *et al*. (2004) red cells indices; MCV and MCHC are particularly important for the diagnosis of anaemia and lead poison in humans and most animals therefore, the significant differences ($p<0.05$) recorded in their values in this study could be as a result of defense reaction (Ahilan *et al*., 2004).

However, the results of HTC, Hb, MCH and PLT of the fish fed treated diets were relatively similar to the control. This showed that the treated diets did not cause any undue stress to the fish because; blood composition is usually altered during stress, disease and malnutrition conditions as documented by Maheswaran *et al*. (2008).

Conclusion

Although, none of the plant extracts studied with the exception of 4mL *A. boonei* leaf extract brought about remarkable weight gain nor negative growth among fish fed the herbal extracts relative to control diet. Similar observations were recorded for the haematological parameters of *Clarias gariepinus* juveniles. Hence, the results of this study had shown that 4 mL *A. boonei* leaf extract could be used to supplement the diet of *C. gariepinus* for optimum growth and improved diseases resistance without being detrimental to the wellbeing of the fish.

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