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Evaluation of Growth, Haematological, Biochemical and Oxidative Stress Parameters of *Clarias gariepinus* Fed with *Alstonia boonei* and *Mitracarpus scaber*

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Abstract

Synthetic agents as growth promoters in aquaculture has become unpopular, hence, the need for better alternatives. This study was conducted to investigate the effects of dietary plants on growth performance, haematological, biochemical and oxidative stress parameters in African catfish. Fish were fed on basal diets for 84 days, the control and six other experimental diets containing different levels of *Alstonia boonei* (0.5%,1.0% and 1.5%) and *Mitracarpus scaber* (0.5%,1.0% and 1.5%) of the basal diets. Fish were weighed bimonthly, blood samples were collected and analyzed. At the end of the experiment, the final weight (FW), weight gain (WG), Average Daily Growth Rate (ADGR) and Specific Growth Rate (SGR) of fish fed with *A. boonei* and *M. scaber* were significantly higher than that of control ($P<0.05$). The values of RBC and haemoglobin of the *M. scaber* (0.5%) group were significantly higher than the other groups including the control. The values of Heterophil-Lymphocyte Ratio (HLR) and Platelet-Lymphocyte Ratio (PLR) in the control group were significantly higher than those of the treatment groups. Biochemical parameters and oxidative stress markers did not show any significant difference between the treatment groups and the control ($P>0.05$). The findings clearly indicated that the plants enhanced growth performance in fish with little or no deleterious effects.

Introduction

Aquaculture is a global food producing sector that is pronto growing and is characterized by intensification in almost all regions of the world (FAO, 2020). This growth has not met the increased demand for aquatic fish food orchestrated by the constant increase in global population. This in part is due to challenges posed to production management among others (Mohammadi *et al.,* 2020). Numerous aquatic organisms both in fresh and marine waters are cultured globally, but African catfish (*Clarias gariepinus*) is an important cultured freshwater fish in terms of production and high disease resistance in several countries (Li *et al*., 2019).

African catfish (*Clarias gariepinus*) was regarded as one of the most suitable species of aquaculture in Africa and has been considered to hold great prospects for fish farming which in turn contributes to nations' economy (Dauda *et al*., 2018). In sub-saharan Africa, *Clarias gariepinus* has replaced tilapia as the most produced fish in aquaculture since 2004 (FAO, 2012).

It is important to device means of increasing production of this fish within the possible shortest period hence, a cost effective and efficient approach is expedient. The use of phytogenic agents has been proven to be relevant in this regards (Rochfort *et al*., 2008). Plant products had been identified to improve nutrients digestibility and availability which result to an

increase in feed conversion ratio and subsequently a higher protein synthesis (Jahazi *et al*., 2020; von Danwitz & Schulz, 2020). An economical way of achieving optimal growth is by altering the quantity of feed consumed by the animal. This is achieved by increasing the palatability of the feed, which is done by adding feed stimulants such as plant additives. Palatability is a limiting factor in the development of artificial diet for aquatic organisms because it indirectly affects acceptability, consumption and digestibility(Al-souti *et al*., 2019). Phytogenic extracts have also been reported to bring about reduction in the cost of treatment, and because they are more biodegradable than the synthetic molecules, they constitute a more environmentally friendly approach and are less likely to produce drug resistance (Hayatgheib *et al*., 2020). To this end, the research into plant extracts candidates for cost effective aquaculture production is expedient. There are numerous plants that have been reported worldwide to possess antimicrobial activities and other beneficial medicinal values (Awotedu *et al*., 2021), among these plants that are of African origin are *Alstonia boonei* (Ogueke *et al*., 2014) and *Mitracarpus scaber* (Adeshina *et al*., 2019).

Alstonia boonei is a large deciduous tree that occurs in moist low land forest but may extend into drier type and can tolerate a wide range of sites. It is widely distributed in many African countries (Orwa *et al*., 2009). The plant is very useful, due to its various ethno-medicinal, pharmacological, toxicological and chemical properties (Adotey *et al*., 2012). All parts of the plant such as leaves, root, stem bark and inflorescences can be of medicinal value (Babatunde, 2017). It has been indicated that *A. boonei* extract may possess potent neuroprotective agents (Ikechukwu *et al*., 2021). It has also been reported that the plant to possess anti-inflammatory, analgesic and antipyretic activities (Olajide *et al*., 2000; Afolabi & Abejide, 2021) and contains bioactive components that aid absorption from the intestine(Oshomoh & Imoyera, 2018). Hence, contributing to body growth of animal. Meanwhile, there is dearth of information about its use in aquaculture which gives credence to this present study.

Mitracarpus scaber on the other hand is one of the medicinal plants commonly found in subtropical regions (Cimanga *et al*., 2004). The extracts of the leaves of this plant have been reportedly suitable for use in aquaculture, with no deleterious effects on growth and blood indices and may be considered as feed supplements for growth promotion, immunity enhancement and reduction in disease susceptibility in fish (Adeshina *et al.,*2019). When plants are suitable in their natural form to enhance growth performance in fish, it is expedient to evaluate their effects on the health status of the fish which can be determined by the analyses of the blood indices.

The use of haematological parameters to evaluate the health status of aquatic animals has been well established (Adamu and Solomon, 2015; Mohammadi *et al*., 2020; Adeshina *et al*., 2021; Afolabi & Abejide, 2021). This study therefore, sought to evaluate the effects of the feed incorporated with these plants on growth enhancement, its haematological and biochemical responses and oxidative stress markers in *Clarias gariepinus*.

Materials and Methods

Plant Additive Diet Preparation

Fish feed ingredients (Table 1) were procured from a reputable commercial fish feed store (Gilgal Fish LTD, Ilorin) and the basal diet was prepared using Pearson Square Method. According to each treatment of the experiment, the single cell protein was added to the experimental feed. The percentages (0.5%, 1% and 1.5%) of the plant leaves were added to each of the experimental feed to make a total of 100% per treatment. The control feed was without any plant additives. The dry ingredients of the feed were thoroughly grinded, mixed and eventually pelletized by addition of water and a binder (starch). The paste formed were extruded through a mincing (manual) machine, with a 2mm die, sun dried and stored in plastic bags until ready for use. The proximate analyses of the experimental diets were taken (Table 2).

Table 1. Feed Formulation

Ingredients	Control	*A. boonei* (%)			*M. scaber*(%)		
	-	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%
Cupid	15	15	15	15	15	15	15
Blood meal	10	10	10	10	10	10	10
Soybean	25	25	25	25	25	25	25
Bone meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Maize+wheat	20	20	20	20	20	20	20
GNC	23	22.5	22.0	21.5	22.5	22.0	21.5
Premix	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vit. C	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Plant	-	0.5	1.0	1.5	0.5	1.0	1.5
Total	100	100	100	100	100	100	100

Proximate Analysis

Feed samples were chemically analyzed in accordance with the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C., 2005). All analyses were carried out in duplicates.

Experimental Design

Four hundred and twenty *Clarias gariepinus* juveniles with an average live weight of 20g were purchased from a reputable fish farm (Teejay feeds and fisheries Nig. Ltd). The fish were transported in a well oxygenated bag half filled with water to the aquaculture unit, faculty of Veterinary Medicine, University of Ilorin. The average weights of fish were taken and recorded before the start of the experiment. The fish were acclimatized for two weeks and fed with commercial diet. All experiments were reviewed and carried out in conformity with the local and international standards of animal care and welfare, and of the Animal Care and Use Research Ethics Committee of University of Ibadan with the assigned number (UI-ACUREC/052-0521/26).

Table 2. Proximate analysis of the feed (g/kg diet)

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)	*M. scaber* (1.0%)	*M. scaber* (1.5%)
Dry Matter	89.00±0.07^{a}	88.92±0.10^{a}	88.92±0.20^{a}	88.62±0.26^{a}	89.80±0.26^{b}	89.20±0.32^{a}	89.30±0.23^{a}
Moisture Content	10.61±0.23^{a}	11.14±0.11^{a}	11.01±0.11^{a}	11.11±0.11^{a}	10.44±0.32^{a}	10.42±0.38^{a}	10.44±0.23^{a}
Crude Protein	38.92±0.01^{b}	41.55±0.01^{c}	40.24±0.01^{b}	40.02±0.01^{b}	37.84±0.02^{a}	38.02±0.01^{a}	37.88±0.10^{a}
Crude Fat	14.95±0.02^{a}	16.49±0.01^{c}	16.51±0.16^{c}	16.30±0.28^{c}	15.11±0.01^{b}	15.10±0.10^{b}	15.11±0.10^{b}
Crude Fibre	14.96±0.01^{b}	11.48±0.01^{a}	11.48±0.18^{a}	11.48±0.11^{a}	15.11±0.01^{c}	15.11±0.30^{c}	15.15±0.11^{c}
Total Ash	12.76±0.01^{a}	14.60±0.01^{c}	14.60±0.22^{c}	14.60±0.21^{c}	13.28±0.22^{b}	13.28±0.19^{b}	13.28±0.32^{b}
Nfe	18.41±0.04^{b}	15.88±0.01^{a}	15.87±0.22^{a}	15.87±0.20^{a}	18.66±0.24^{b}	18.66±0.20^{b}	18.66±0.21^{b}

*Different letters as superscripts across the rows indicate significant differences (P<0.05)

Table 3. Growth performance of fish fed with feed incorporated with *Alstonia boonei* and *Mitracarpus scaber* respectively for 84 days

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)	*M. scaber* (1.0%)	*M. scaber* (1.5%)
IW (g)	20.43±0.12^{a}	20.53±0.15^{a}	20.67±0.09^{a}	20.37±0.12^{a}	20.87±0.18^{a}	20.40±0.10^{a}	20.67±0.03^{a}
FW (g)	71.23±0.56^{a}	88.08±0.20^{e}	81.39±1.23^{c}	76.41±0.68^{b}	101.77±0.42^{f}	85.58±0.43^{d}	83.79±0.45^{d}
WG (g)	50.79±0.47^{a}	67.54±0.29^{f}	60.72±1.32^{c}	56.04±0.66^{b}	80.90±0.41^{g}	65.18±0.47^{e}	63.12±0.45^{d}
WG (%)	248.58±1.68^{a}	328.99±3.57^{d}	293.87±7.62^{c}	275.20±3.49^{b}	387.76±4.05^{e}	319.52±3.32^{d}	305.42±2.29^{c}
ADGR (g/day)	0.60±0.01^{a}	0.80±0.00^{f}	0.72±0.02^{c}	0.67±0.01^{b}	0.96±0.00^{g}	0.78±0.01^{e}	0.75±0.01^{d}
SGR (%/day)	1.49±0.01^{a}	1.73±0.01^{d}	1.63±0.02^{c}	1.57±0.01^{b}	1.89±0.01^{e}	1.71±0.01^{d}	1.67±0.01^{c}
FCR	1.60±0.01^{c}	1.21±0.01^{b}	1.36±0.01^{bc}	1.45±0.01^{bc}	1.03±0.01^{a}	1.25±0.01^{b}	1.30±0.01^{b}

*Different letters as superscripts across the rows indicate significant differences (P<0.05)

Table 4. Haematological parameters at week 4

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)	*M. scaber* (1.0%)	*M. scaber* (1.5%)
RBC (×10^{6})	4.82±0.38^{a}	4.40±0.43^{a}	4.32±0.04^{a}	3.72±0.31^{a}	4.27±0.50^{a}	3.09±0.46^{a}	4.48±0.50^{a}
HGB (g/dl)	9.49±0.82^{a}	8.82±0.81^{a}	8.60±0.68^{a}	7.53±0.62^{a}	8.47±0.98^{a}	5.96±0.98^{a}	8.66±0.98^{a}
PCV (%)	29.67±2.26^{a}	27.11±2.57^{a}	27.78±2.41^{a}	23.22±1.88^{a}	26.33±2.96^{a}	19.00±2.79^{a}	28.00±2.87^{a}
MCV (fl)	60.67±0.17^{a}	61.44±0.18^{b}	61.00±0.24^{ab}	61.44±0.29^{b}	61.44±0.24^{b}	62.33±0.24^{c}	61.67±0.29^{bc}
MCH (pg)	20.00±0.09^{a}	19.40±0.46^{a}	19.69±0.33^{a}	20.28±0.05^{a}	19.90±0.35^{a}	19.57±0.50^{a}	19.46±0.48^{a}
MCHC (g/dl)	32.86±0.04^{a}	31.48±0.76^{a}	32.32±0.60^{a}	32.71±0.07^{a}	32.19±0.58^{a}	31.06±0.78^{a}	31.37±0.81^{a}
WBC (×10^{3})	5.58±0.35^{a}	7.41±1.23^{a}	6.57±0.86^{a}	6.49±0.66^{a}	8.34±0.91^{a}	7.67±0.91^{a}	6.87±0.60^{a}
HET (×10^{3})	2.49±0.16^{a}	3.27±0.71^{a}	2.94±0.35^{a}	2.99±0.27^{a}	3.68±0.42^{a}	3.64±0.49^{a}	3.03±0.80^{a}
LYM (×10^{3})	2.91±0.28^{a}	3.93±0.58^{a}	3.43±0.58^{a}	3.28±0.38^{a}	4.29±0.52^{a}	3.73±0.41^{a}	3.67±0.43^{a}
EOS (×10^{3})	0.03±0.02^{a}	0.07±0.02^{a}	0.03±0.01^{a}	0.06±0.01^{a}	0.12±0.03^{a}	0.08±0.05^{a}	0.04±0.02^{a}
MON (×10^{3})	0.15±0.02^{a}	0.14±0.02^{a}	0.16±0.03^{a}	0.12±0.02^{a}	0.28±0.04^{a}	3.21±0.2.97^{a}	0.19±0.02^{a}
PLT (×10^{3})	177.78±6.33^{a}	148.88±9.54^{a}	154.67±10.14^{a}	158.11±9.60^{a}	171.67±12.9^{a}	177.11±6.93^{a}	162.11±5.68^{a}
HLR	0.91±0.10^{a}	0.82±0.11^{a}	1.00±0.14^{a}	0.95±0.04^{a}	0.91±0.11^{a}	0.60±0.06^{a}	0.90±0.09^{a}
PLR	64.77±5.54^{a}	44.24±5.69^{a}	61.36±14.38^{a}	54.56±7.39^{a}	44.21±5.81^{a}	53.66±7.18^{a}	50.06±5.92^{a}

*Different letters as superscripts across the rows indicate significant differences (P<0.05). RBC (red blood cell); HGB (haemoglobin); PCV (packed cell volume); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration); WBC (white blood cell); HET (heterophil); LYM (lymphocyte); EOS (eosinophil); MON (monocyte); PLT (platelet); HLR (heterophil lymphocyte ratio); PLR (platelet lymphocyte ratio).

The fish were distributed equally into six experimental treatment groups with a control in triplicates with 20 juvenile African catfish (*Clarias gariepinus*) in each circular plastic aquarium with dimensions of 50cm x 34cm x 27cm of 40 litres capacity of water totaling twenty-one plastic aquaria for the experimental set-up. A total of four hundred and twenty juvenile African catfish were used and fed 4% of their body weight twice daily.

The feeding regimen was done in the morning and evening at 8am and 5pm GMT +1 respectively. Fish weights were taken every two weeks and the feed was adjusted accordingly to 4% of the body weight.

The source of water was from University of Ilorin water station and each experimental tank was well aerated using air stone and aerator pumps. Water quality parameters such as temperature (°C), dissolved oxygen (DO) and pH were measured weekly and maintained at 26.98±0.03°C, 5.17±0.01 mg/L and 7.13±0.02 respectively. Dissolved oxygen and temperature were measured in-site using a portable oxygen meter (Jenway, London, UK), while pH meter (Digital Mini-pH Meter, USA) was used for the measurement of pH. The study followed a 2x3 factorial experiment in a Completely Randomised Design (CRD) for twelve weeks.

Growth Performance Evaluation

Twenty fish per each tank were collected, group-weighed bimonthly on a digital ScoutPro sensitive scale (Model: KD-200-110, USA) and the average was determined. At the end of the feeding trial, the parameters of growth performance were calculated as:

Body weight gain (WG) in (g) = FW – IW

Weight gain (WG) in (%) = WG / IW x 100

Average daily growth rate (ADGR; g/day) = WG / No. of feeding days

Specific growth rate (SGR; % / day) = Ln FW – Ln IWx100 / Length of the culture period

Feed conversion ratio (FCR) = feed intake (g) / weight gain (g)

Where FW = final mean weight; IW = initial mean weight; WG = weight gain; Ln = log with a base of e.

Haematological and Oxidative Stress Evaluation

Blood Collection

Prior to sampling, fish were fasted for 24 h and moderately sedated with sodium bicarbonate buffered tricaine methanesulfonate (MS222, 30 mg/L, Syndel, Ferdale, Washington, USA) for 5 min(Adeshina *et al*., 2021). Blood samples were collected from eight African catfish from each replicate of the treatment and control from the caudal vein using 23G needle with 2ml hypodermic syringe. The blood samples for haematological analysis were collected into lithium heparinized tubes on weeks 4, 8 and 12 and non-heparinized blood on only week 12. The blood samples were put on ice and transported to the laboratory for the analyses.

Haematology

The red blood cell (RBC) and white blood cell (WBC) counts were determined using an improved Neubauer

Table 5. Haematological parameters at week 8

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)	*M. scaber* (1.0%)	*M. scaber* (1.5%)
RBC (×10^6)	5.82±0.52^a	5.38±0.35^a	5.13±0.45^a	5.38±0.56^a	4.93±0.42^a	5.07±0.29^a	5.79±0.43^a
HGB (g/dl)	9.53±0.1.81^a	10.83±0.72^a	10.33±0.89^a	10.85±1.11^a	9.93±0.84^a	10.23±0.59^a	11.67±0.87^a
PCV (%)	34.00±3.42^a	33.00±2.13^a	31.50 ±2.68^a	33.00±3.35^a	30.33±2.51^a	31.17±1.76^a	35.50±2.60^a
MCV (fl)	60.83±0.17^a	61.00±0.00^a	60.83±0.17^a	61.17±0.17^a	61.17±0.17^a	61.00±0.00^a	61.00±0.00^a
MCH (pg)	20.18±0.04^a	20.20±0.26^a	20.05±0.09^a	20.15±0.02^a	20.13±0.06^a	20.15±0.02^a	20.12±0.02^a
MCHC (g/dl)	32.93±0.10^a	32.88±0.05^a	32.73±0.15^a	32.77±0.10^a	32.83±0.03^a	33.05±0.15^a	32.87±0.07^a
WBC (×10^3)	9.03±1.54^a	8.22±1.05^a	9.10±1.75^a	8.21±0.90^a	9.62±1.01^a	7.54±0.82^a	7.68±0.94^a
HET (×10^3)	3.93±0.67^a	3.09±0.28^a	4.25±0.95^a	3.34±0.62^a	4.10±0.37^a	2.77±0.41^a	3.90±0.40^a
LYM (×10^3)	4.79±0.93^a	4.87±0.82^a	4.62±0.80^a	4.61±0.46	5.17±0.64^a	4.52±0.48^a	3.45±0.60^a
EOS (×10^3)	0.06±0.03^a	0.06±0.03^a	0.06±0.03^a	0.00±0.00^a	0.07±0.03^a	0.01±0.01^a	0.09±0.04^a
MON (×10^3)	0.27±0.04^a	0.20±0.04^a	0.17±0.03^a	0.26±0.06^a	0.28±0.05^a	0.24±0.04^a	0.24±0.07^a
PLT (×10^3)	189.50±7.30^a	205.17±12.07^a	208.83±20.06^a	194.33±9.65^a	220.50±20.6^a	198.50±7.97^a	192.00±12.59^a
HLR	0.84±0.09^a	0.70±0.09^a	0.88±0.07^a	0.73±0.14^a	0.82±0.06^a	0.62±0.07^a	1.24±0.16^b
PLR	46.49±6.44^a	55.45±8.73^a	49.62±6.08^a	43.82±4.03^a	45.85±6.05^a	46.43±5.04^a	62.02±8.12^a

*Different letters as superscripts across the rows indicate significant differences (P<0.05). RBC (red blood cell); HGB (haemoglobin); PCV (packed cell volume); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration); WBC (white blood cell); HET (heterophil); LYM (lymphocyte); EOS (eosinophil); MON (monocyte); PLT (platelet); HLR (heterophil lymphocyte ratio); PLR (platelet lymphocyte ratio).

haemocytometer. Hematocrit (Hct) was measured using the standard microhaematocrit method and reported in percentage. Haemoglobin concentration (Hb) were determined by the cyanmethaemoglobin spectrophotometry method (Blaxhall & Daisley, 1973). The erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe (2008). MCV = PCV x 10/RBC in fentolitre, MCH = Hb x 10/RBC in pictograms and mean MCHC = Hb x 100/PCV in g/dl (Adamu and Solomon, 2015). The differential leukocyte counts were obtained from May-Grunwald-Giemsa stained blood smears (Sepperumal & Saminathan, 2013).

Heterophil lymphocyte ratio (HLR) is a proportion obtained by dividing the absolute peripheral blood cell count of heterophils by that of the lymphocytes while Platelet lymphocyte ratio (PLR) is the value obtained from the division of platelet count by the absolute value of the lymphocyte.

$$\text{HLR} = \frac{\text{Absolute peripheral blood cell count of heterophil}}{\text{Lymphocyte}}$$

$$\text{PLR} = \frac{\text{Platelet count}}{\text{Lymphocyte}}$$

Oxidative Stress Markers Analysis

Serum samples for biochemical analyses were centrifuged at 3000 rpm for 10 minutes with Hawsley bench centrifuge (P specra, Centromix no 231254 CD7000549, Spain). The samples were stored at -20^{o}C until used for the analyses. The activity levels of superoxide dismutase (SOD), malonaldehyde (MDA), Glutathione-S-transferase (GST), Glutathione peroxidase (GPx), Myeloperoxidase (MPO) and catalase were measured in the serum from fish in each group using commercially available standard kits (Nanjing Jiancheng Bioengineering Co. Ltd., China), following the manufacturer's instructions with mild modification by Ma *et al*. (2014).

Biochemical Analysis

The blood samples for serum biochemical tests were allowed to clot at room temperature for 30 minutes and then centrifuged at 3000 rpm for 15 minutes; sera were carefully harvested into labelled vials and then stored at -20^{o}C until analyzed. The samples were used to measure the concentrations of total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine (Cr) and cholesterol as well as the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) using commercial test kits (Agappe, India) using a digital ultraviolet spectrophotometer according to method described by (Ma *et al*., 2014).

Statistical Analysis

The data obtained were recorded in excel sheet and subjected to one-way analysis of variance (ANOVA) using IBM statistical package (SPSS version 20) to determine differences among the treatments and control in all parameters. Individual means were separated using Duncan multiple range test. All data were presented as means ± SE, and were reported as significant at $P<0.05$ according to Dytham (2011).

Results

Proximate Analysis

Table 2 revealed that the feed with *M. scaber* (0.5%) had significant higher dry matter and crude fibre than the control and feed with *A. boonei.* Moisture content was not significantly different between the treatment feeds and control. The feed with *A. boonei*

Table 6. Haematological parameters at week 12

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)
RBC(×10^6)	3.64±0.17^{ab}	4.88±0.15^{bc}	5.21±0.73^{bc}	4.02±0.41^{abc}	5.77±0.07^{c}
HGB (g/dl)	6.90±0.50^{ab}	9.77±0.23^{bc}	10.30±1.50^{bc}	8.80±1.15^{abc}	11.30±0.06^{c}
PCV (%)	21.33±1.33^{ab}	29.67±0.67^{bc}	32.33±4.33^{bc}	26.00±3.06^{abc}	34.67±0.33^{c}
MCV (fl)	60.33±0.33^{a}	60.67±0.67^{a}	61.00±0.00^{a}	61.00±0.58^{a}	60.00±0.00^{a}
MCH (pg)	19.97±0.33^{a}	19.80±0.20^{a}	20.17±0.03^{a}	18.83±0.91^{a}	19.70±0.21^{a}
MCHC (g/dl)	32.33±0.28^{a}	32.17±0.67^{a}	32.80±0.12^{a}	31.07±1.74^{a}	32.47±0.37^{a}
WBC (×10^3)	12.00±0.00^{b}	8.71±0.75^{a}	7.85±1.70^{a}	7.45±0.22^{a}	8.05±1.29^{a}
HET (×10^3)	6.94±0.42^{c}	3.81±0.47^{ab}	3.64±1.05^{a}	2.89±0.01^{a}	3.49±0.42^{a}
LYM (×10^3)	4.88±0.49^{a}	4.67±0.32^{a}	3.99±0.64^{a}	4.20±0.15^{a}	4.49±0.86^{a}
EOS (×10^3)	0.00±0.00^{a}	0.09±0.01^{a}	0.03±0.03^{a}	0.00±0.00^{a}	0.03±0.03^{a}
MON (×10^3)	0.18±0.09^{a}	0.14±0.04^{a}	0.25±0.04^{a}	0.19±0.03^{a}	0.09±0.01^{a}
PLT (×10^3)	387.00±0.7.02^{c}	292.00±6.35^{b}	208.00±8.72^{a}	217.00±31.79^{ab}	230.67±51.02^{ab}
HLR	1.46±0.23^{b}	0.81±0.05^{a}	0.87±0.13^{a}	0.69±0.02^{a}	0.81±0.11^{a}
PLR	81.09±9.26^{c}	63.34±5.22^{abc}	54.05±6.22^{ab}	51.38±6.16^{a}	50.79±1.86^{a}

*Different letters as superscripts across the rows indicate significant differences ($P<0.05$). RBC (red blood cell); HGB (haemoglobin); PCV (packed cell volume); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration); WBC (white blood cell); HET (heterophil); LYM (lymphocyte); EOS (eosinophil); MON (monocyte); PLT (platelet); HLR (heterophil lymphocyte ratio); PLR (platelet lymphocyte ratio).

(0.5%) had significant higher crude protein, crude fat and total ash than the control and the other treatment feed. Meanwhile, there was no significant difference in the content of non-fat ester (NFE) between the feed with *M. scaber* and control but significant differences existed between the two above and the feed with *A. boonei*.

Growth Performance

The final weight (FW), weight gain (WG), ADGR and SGR of fish fed with *A. boonei* and *M. scaber* were statistically higher than the control diet. The group fed with *M. scaber* (0.5%) had the highest weight gain, followed by *A. boonei* (0.5%), while the control diet group had the least (Table 3). These parameters significantly increased in the treatment groups in comparison with the control (P<0.05).

Haematological Parameters

At week 4, there was no significant difference across all the haematological parameters between the treatment and control groups except the MCV of the *M. scaber* (1.0%) group with highest value of 62.33±0.24fl and the control with the lowest value of 60.67±0.17fl (Table 4). At week 8, there was no significant difference in all the haematological parameters between the treated groups and the control (Table 5). At week 12, the values of RBC and haemoglobin of the *M. scaber* (0.5%) group were significantly higher than the other groups including the control, but the *M. scaber* (1.0%) group has the lowest values. There is no significant difference among the different concentrations of *A. boonei* and the difference between the *A. boonei* group and control is also not significant. The values of HLR and PLR in the control group are significantly higher than those of the treatment groups (Table 6).

Serum Analyses and Oxidative Stress Markers

The globulin, albumin-globulin ratio, creatinine, BUN and cholesterol levels did not show any significant difference between the treatment groups and the control (P>0.05). The protein level in the control group was significantly higher (P<0.05) than the *M. scaber* (1.5% and 1.0%) and *A. boonei* (1.5%) but not significantly greater than the rest of the treatment groups. *M. scaber* (1.5%) had the lowest albumin level. There was no significant difference in the values of AST and ALT across the treatment groups and the control. Meanwhile, *M. scaber* (1.5%) had the significant ALP values lower than other treatment groups as well as the control (Table 7). There was no significant difference (P>0.05) in the activity of SOD between the control and *A. boonei* (1.0%). Meanwhile, there was significant difference between the above groups and *M. scaber* (1.0% and 1.5%) where *M. scaber* (1.5%) had the lowest activity of SOD. There were no significant differences in the activities of catalase, glutathione peroxidase (GPX), glutathione S-transferase (GST) and malanodialdehyde (MDA) among all the treatment groups and the control. However, catalase in *A. boonei* (0.5%) had the highest activity, when compared to the control with the lowest activity. There was no significant difference in the activity of MPO between *A. boonei* (0.5%) and *M. scaber* (1.0%) but a significant difference existed between the above two treatment groups and other groups including the control. The group *M. scaber* (1.5%) having the lowest activity (Table 8).

Discussion

The use of *A. boonei* and *M. scaber* in aquaculture has not been extensively imbibed, inspite of their proven medicinal values in terrestrial animals and man. Meanwhile, plants with medicinal benefits have broadly attracted researchers of aquaculture interest due to their innate potential to improve growth, feed digestibility, immune responses, and reduce antibiotic resistance and decrease drug residue (Adeshina *et al*., 2019; Mehrinakhi *et al*., 2021). The feed mixed with the study plants had significant improved proximate analysis compared to the control. This may contribute to the growth enhancement in the treatment groups more than the control. This finding is supported by the earlier report of (Adeshina *et al*., 2019) that *Mitracarpus scaber* leaves extract (MSLE) included in a diet contributed significantly to the growth performance and efficient nutrient utilization and enhanced innate

Table 7. Serum biochemical parameters at week 12

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)	*M. scaber* (1.0%)	*M. scaber* (1.5%)
ALP (U/L)	50.80±1.89^{a}	48.69±0.95^{a}	51.47±0.83^{a}	48.88±0.88^{a}	47.48±0.69^{ab}	49.43±0.25^{a}	43.20±3.30^{b}
AST (U/L)	64.32±6.04^{a}	59.77±7.01^{a}	63.75±2.77^{a}	60.26±2.08^{a}	64.17±5.28^{a}	58.14±4.80^{a}	64.52±0.87^{a}
ALT (U/L)	71.40±2.71^{a}	78.98±5.94^{a}	63.63±2.62^{a}	64.54±3.68^{a}	67.26±8.44^{a}	60.80±4.95^{a}	62.21±1.60^{a}
PROTEIN (g/dL)	10.26±1.20^{a}	8.19±0.89^{ab}	8.52±1.28^{ab}	6.62±0.42^{a}	8.34±0.52^{ab}	7.06±0.23^{a}	6.48±0.40^{a}
ALB (g/dL)	2.20±0.21^{b}	2.27±0.29^{b}	2.28±0.34^{b}	1.67±0.08^{ab}	2.20±0.30^{b}	1.59±0.16^{ab}	1.35±0.14^{a}
GLOB (g/dL)	8.06±1.36^{a}	5.92±0.61^{a}	6.24±0.96^{a}	4.95±0.35^{a}	6.14±0.23^{a}	5.46±0.07^{a}	5.13±0.50^{a}
AGR	0.29±0.06^{a}	0.38±0.02^{a}	0.37±0.02^{a}	0.34±0.01^{a}	0.36±0.04^{a}	0.29±0.03^{a}	0.27±0.05a
CREAT (mg/dL)	41.33±8.82^{a}	61.33±17.64a	48.67±12.97a	38.00±0.10^{a}	31.33±6.67a	38.00±5.77^{a}	28.00±5.77^{a}
BUN (mg/dL)	6.71±1.54^{a}	6.39±0.79^{a}	6.73±1.00^{a}	5.13±0.26^{a}	5.78±0.56^{a}	5.70±0.98^{a}	7.34±0.48^{a}
CHOL (mg/dL)	134.13±15.58^{a}	145.59±13.24^{a}	127.76±11.23^{a}	121.82±2.58^{a}	125.21±3.70^{a}	113.09±6.85^{a}	119.27±5.32^{a}

*Different letters as superscripts across the rows indicate significant differences (P<0.05). ALP (alkaline phosphatase); AST (aspartate aminotransferase); ALT (alanine aminotransferase); ALB (albumin); GLOB (globulin); AGR (albumin globulin ratio); CREAT (creatinine); BUN (blood urea nitrogen); CHOL (cholesterol).

immunity in common carp. It is evident in this study that feed fortified with *A. boonei* and *M. scaber* improved the weight gain in African catfish, thus contributing to the increased growth performance in these fish. The feeds with the lowest percentages of plant additives i.e. *M. scaber* (0.5%) and *A. boonei* (0.5%) recorded the highest weight gain in the fish. Hence, this percentage in both plants would be adequate as feed additives to enhance growth performance. The enhancement of growth performance in fish by the application of plant additives have been broadly studied and reported (Adeshina *et al*., 2019; Mehrinakhi *et al*., 2021; Mohammadi *et al*., 2020; Sadeghi *et al*., 2020). The benefits of phytogenic agents in growth enhancement and feed digestibility in fish could be associated with their role in the growth of beneficial resident microorganisms leading to enhanced feed intake and improved weight gain (Mehrinakhi *et al*., 2021). Growth rate and weight gain are directly associated with the capability of animal to ingest, digest and absorb nutrients present in the feed. The earlier study of Adeshina *et al*. (2019) reported the improved growth and efficient feed utilization of common carp (*Cyprinus carpio*) fed with *Mitracarpus scaber* leaves extract (MSLE) which was attributed to higher consumption of *M. scaber* containing feed. Adeshina *et al*. (2019a) also reported that the p-cymene and eugenol present in *M. scaber* could be associated with its acceptability by the fish. Abdel-Tawwab and El-Araby (2021) also attributed the growth promoting activity of licorice to its high contents of various bioactive compounds such as flavonoids, saponins, isoflavonoids, phenols among others which are also present in these study plants. These compounds were also reported to enhance digestion of nutrient leading to improved growth. Although several studies have been carried on the antimicrobial effects and other benefits of *A. boonei* in terrestrial animals (Afolabi & Abejide, 2021; Awotedu *et al*., 2021; Ikechukwu *et al*., 2021; Ogueke *et al*., 2014; Olajide *et al*., 2000), there is dearth of reliable information on its effects on growth enhancement in fish and this gives credence to the importance of this study. Meanwhile, Akinmoladun *et al*. (2007) reported that *A. boonei* contained some vitamins and macro-elements which may also be essential for the growth enhancement in fish. The ban of antibiotics as growth promoters in some countries (Rochfort *et al*., 2008) and the need for an alternative, with minimal or without negative effect have given credence to the application of plant additives in modern aquaculture.

Haemato-biochemical indices have been a valuable and acceptable tool used in determining the health status of fish. It was expedient to evaluate the effects of the current study plants on these indices to ascertain their suitability for growth enhancement without any deleterious effect on the fish health. Haematological parameters are an essential diagnostic aid for the assay of physiological and pathological changes in fish (Fazio, 2018). At weeks 4 and 8 of the experiment, there was no significant haematological alteration between the treatment and control groups. Similar to this finding, is a study that observed that dietary Moringa leaves fed at different concentrations did not show any significant haematological alterations with the control in Bocourti's catfish (El-gawad *et al*., 2019). At week 12, the group *M. scaber* (0.5%) had the highest values of RBC and haemoglobin, which is an indicator of an improved health status by increased tissue oxygenation. This result is similar to the earlier report of Adeshina *et al*. (2021), that Nile tilapia fed with *Mitracarpus scaber* leave extract (MSLE) revealed higher values erythrocyte, haemoglobin and PCV than the control. *A. boonei* was also reported to contain high iron content which could help improve haematological parameters in fish (Akinmoladun *et al*., 2007).

Haematological ratio is also an important prognostic and diagnostic tool used in assessing the state of health of an animal. In this study, it was revealed that by the end of week 12 of the feeding regimen, the HLR and PLR in the control group were higher than the treatment groups, indicating that the plant additives were suitable for use in the fish. The lower the ratios, the good the prognosis as reported in earlier studies (Berckelaer *et al*., 2020; Hematol *et al*., 2015; Ulas *et al*., 2015). The non-significant differences in the value of BUN and creatinine between the treatment groups and the control are indications that the plant additives do not pose any adverse effect on the kidney functions. This is in agreement with the previous study reported by Abdel-wahab *et al*. (2021) on MSLE in Nile tilapia (*Oreochromis niloticus*).

ALT and AST, and ALP are cytosolic and induced enzymatic activities respectively used in evaluating the functions of the liver in the fish. In this present study, it was revealed that there was no significance difference in the values of the ALT and AST between the treatment groups and the control and the value of ALP is higher in

Table 8. Oxidative stress markers at week 12

Parameters	Control	*A. boonei* (0.5%)	*A. boonei* (1.0%)	*A. boonei* (1.5%)	*M. scaber* (0.5%)	*M. scaber* (1.0%)	*M. scaber* (1.5%)
SOD (IU/L)	2.20±0.29^{c}	1.29±0.30^{ab}	2.22±0.29^{c}	1.58±0.14^{abc}	1.91±0.25^{bc}	1.13±0.24^{a}	0.86±0.15^{a}
GST (IU/L)	0.08±0.04^{a}	0.08±0.03^{a}	0.06±0.02^{a}	0.05±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
MPO (IU/L)	897.80±52.18^{ab}	2273.74±82.30^{c}	1060.57±113.93^{ab}	1393.75±167.47^{abc}	1805.77±295.59^{bc}	2243.22±132.16^{c}	544.27±67.72^{a}
MDA (IU/L)	8.87±0.56^{a}	6.00±1.5^{a}	7.07±1.77^{a}	4.45±0.23^{a}	5.58±1.00^{a}	5.11±0.56^{a}	2.13±0.56^{a}
GPX (IU/L)	136.88±24.69^{a}	124.50±17.67^{a}	85.50±3.25^{a}	66.75±10.36^{a}	135.00±26.05^{a}	70.88±11.91^{a}	165.37±91.07^{a}
CAT (IU/L)	5.77±1.78^{a}	9.1±1.43^{a}	6.98±1.64^{a}	6.75±1.23^{a}	7.66±1.08^{a}	7.96±0.49^{a}	8.22±0.97^{a}

*Different letters as superscripts across the rows indicate significant differences (P<0.05).

control than in *M. scaber* (1.5%) group. This is an indication that the plant additives in this study may not be hepatotoxic. Unlike in the earlier study of Abdel-tawwab & El-araby (2021) whereby grades of dietary plants caused decrease in the liver enzymes which was associated with hepato-protective potentials of the plant additives. Cultured fish are often exposed to stress, which can result to high morbidity and mortality. There are important enzymes including catalase, superoxide dismutase (SOD), melanodealdehyde, melanoperoxidase, glutathione peroxidase and glutathione S-transferase that are reportedly involved in the maintenance of normal redox homeostasis and improvement of the imbalance in the biological reactive oxygen species (ROS) (Abdel-latif *et al*., 2020; Abdel-tawwab & El-araby, 2021). It is evident in this study that the only a few treatment groups had a significant increase in the values of the oxidative stress markers more than the control. It suffices that the plant additives did not subject the fish to further stress but also improve the anti-oxidant status of the fish. When fish are subjected to stress, reactive oxygen species (ROS) are produced in response to stress. The increased production of this biological agent will elicit the activities of antioxidants to counter the anticipated cellular damage (Wang *et al*., 2013; Arun *et al*., 2018; Chen *et al*., 2020) . Thus, the study plants did not cause excessive production of ROS.

Conclusion

The findings of this study clearly indicate that *Alstonia boonei* (0.5%, 1.0% and 1.5%) and *Mitracarpus scaber* (0.5%, 1.0% and 1.5%) inculcated in formulated fish feed could be used to enhance the growth performance in fish (*Clarias gariepinus*) without any heath challenges evidenced by the haematological parameters and oxidative stress markers. This makes the plants suitable as feed additives in aquaculture.

Ethical Statement

All experiments were reviewed and carried out in conformity with the local and international standards of animal care and welfare, and of the Animal Care and Use Research Ethics Committee of University of Ibadan with the assigned number (UI-ACUREC/052-0521/26).

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Author Contribution

First Author (Abdullateef Ajadi**):** Conceptualization, Methodology, Writing -original draft; Second Author (Emikpe Benjamin): Conceptualization, Formal Analysis, Investigation, Methodology, Supervision; Writing -review and editing; Third Author (Afusat Jagun Jibril): Supervision; Writing -review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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