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GROWTH PERFORMANCES AND IMMUNOLOGICAL INDICES OF CLARIAS GARIEPINUS FINGERLINGS FED DIETS INCORPORATED WITH ALOE VERA LEAVES-PASTE

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Abstract
African mud catfish, Clarias gariepinus, has become an important aquaculture species in Nigeria for its high economic value and cherished delicacy. Proper growth and survival of fish in an aquaculture system depend on good nutrition. This study examined the growth performance and immunological profiles of C. gariepinus fingerlings fed diets incorporated with Aloe vera leaves-paste. The experimental diets were prepared by incorporating basal feed (40.36% crude protein) with Aloe vera leaves-paste at 0.5%, 1.0%, 1.5%, and 2.0% rates. The four diets were ALD1, ALD2, ALD3, and ALD4, respectively. Basal feed without Aloe vera leaves-paste (0%) is the control (CTRL). The proximate compositions of the experimental basal diet were analysed. The diets were fed to C. gariepinus fingerlings for 6 weeks and monitored for growth performances, feed utilisation, and immunological profiles. There were significant differences ($p<0.05$) in the results. The highest mean weight gain (14.73±2.58g/fish), condition factor (0.55), and protein efficiency ratio (0.58±0.09) were recorded for ALD2. The white blood cell count (270.70×103/µl) and red blood cell count (2.08×106/µl) were highest for ALD2. The highest mean cell volume (142.20fl) was recorded for ALD1 and the lowest (28.30fl) for CTRL. Lymphocyte values were not significantly different ($p>0.05$) from the initial value of 98.50% at the start of the feeding trials. Neutrophils were reported to be highest (2.40%) in CTRL and lowest (1.60%) in ALD2. The study concluded that C. gariepinus could be fed diets incorporated with Aloe vera leaves-paste at 1.0% for improved growth and health performances.

Keywords: Aloe vera, Growth Performance, Immunology, Clarias gariepinus, Aquaculture

Introduction

In Nigeria, the aquaculture industry has become a key sector for national food security in approaching the country's growing population and protein demands. The annual fish demand in Nigeria significantly exceeds what the local production can supply, causing the country to depend on imports to bridge the demand-supply gap (FAO, 2020). The domestic fish consumption in Nigeria is over 1.5 million metric tonnes per year, 30% of which can only be met by the local fish production (Erondu *et al*., 2017). Nigeria has continued to rely heavily on import supply to meet the remaining 70% demand (Erondu *et al*., 2017). The socio-economic importance of fish farming provides an avenue to reduce the dependency of the country on imports while creating jobs and generating income for local communities (Selig *et al*. 2018).

One of the key focus of the Nigerian government is to reduce the country's reliance on the importation of fish to achieve self-sufficiency (Adedeji and Adetunji, 2019). The right information and understanding about the structural and production requirements, such as technological investments and access to quality and affordable fish feed, to ensure sustainable development of the aquaculture industry, is essential (Adelekan and Oluwatusin, 2018).

African mud catfish, *Clarias gariepinus*, has become an important aquaculture species in Nigeria for its high economic value and cherished delicacy. Also, its rapid growth rate, high production, and good disease resistance capability in culture systems have contributed to the fish species choice for fish farming in Nigeria. In aquaculture production, fish feeds account for 50 to 70% of the total production costs (Idowu, 2019). Proper growth and survival of fish in a culture system depends largely on good nutrition – fish feed with proper nutritional compositions supplied in the right proportions (Adelekan and Oluwatusin, 2018). Moreover, adequate information is needed on sustainable practices, such as optimising feed resources, reducing environmental impacts of aquaculture, and implementing efficient water management techniques to align the catfish industry with the global development goal of sustainability (El-Sayed, 2021).

There are growing developments in the utilisation of the biochemical richness of medicinal plants for novel applications in the areas of feed ingredients and nutraceuticals (Ugoala *et al*., 2014; Idowu, 2019). Various achievements have been made with medicinal plants in human and veterinary medicines. Though research is ongoing and remarkable advancements are being made, there is still more to be explored in aquaculture (Idowu, 2019). Therefore, more studies exploring the significance of medicinal plants in sustainable aquaculture development in enhancing fish growth performance and immune systems to boost fish production are needed. This present study aimed at examining the growth performance and immunological indices of *C. gariepinus* fingerlings fed diets incorporated with *Aloe vera* leaves-paste.

MATERIALS AND METHODS

Experimental Site

This research was conducted at the fish farm of the Department of Fisheries, Faculty of Agriculture, Modibbo Adama University, Yola, Nigeria, located within latitudes 9°20'–9°33'N and longitudes 12°30'–12°50'E, at an altitude of 185.9m (Mallum, 2016).

Collection and Preparation of *Aloe vera* leaves-paste

Fresh *Aloe vera* leaves were procured from a plant nursery in Jimeta -Yola Metropolis and identified at the Department of Forestry, Modibbo Adama University, Yola. The *Aloe vera* leaves-paste was prepared as described by Muhammad *et al.* (2014). The root parts of the plant were cut off using a clean knife. The leaves were washed thoroughly with water and allowed to stand upright in a bowl to allow the yellow sap from the leaves to drain out. Then the *Aloe vera* leaves were carefully chopped in a blender (TOWER Model T12002) with a clean knife to obtain a blended paste of the leaves.

Processing of Experimental Feed Ingredients

Ingredients comprising fish meal, toasted soybean meal, yellow maize meal, vitamin and mineral premixes, lysine, methionine, palm oil, cassava starch, and salt used in producing the experimental diets were purchased from Jimeta Ultra-modern Market, Yola. The feedstuffs were given appropriate processing and handling to improve digestibility and remove anti-nutritional factors. Soybeans were toasted and milled. The fish and yellow maize were milled separately using a domestic milling machine. Cassava starch was purchased in powdered form and used as a feed binder. The basal feed was formulated according to Idowu (2019).

Formulation of Experimental Diets

The experimental diets were prepared by incorporating basal feed (40.36% crude protein) with *Aloe vera* leaves-paste at 0.5%, 1.0%, 1.5%, and 2.0% rates and designated as ALD1, ALD2, ALD3, and ALD4, respectively. Basal feed without *Aloe vera* leaves-paste served as the control (0%) and was designated as (CTRL). The percentage ingredient composition of the experimental feed is shown in Table 1.

Table1. Percentage composition of experimental basal feed with 40.36% crude protein

Ingredients	Composition (% dry matter)
Fish meal	32.00
Soya bean	33.00
Yellow Maize	30.00
Vitamin premixes	1.0
Mineral premixes	0.5
Lysine	0.5
Methionine	0.5
Palm oil	1.0
Cassava starch	1.0
Common salt	0.5
Total	100.0
Calculated crude protein	39.94

The proximate compositions of the experimental basal diet were carried out at the Dry Laboratory of the Department of Fisheries, Modibbo Adama University, Yola, according to the Association of Official Analytical Chemist Methods (AOAC, 2000).

Experimental Fish and Feeding Trial

A total of 150 apparently healthy *C. gariepinus* fingerlings were procured from a reputable commercial fish farm in Yola. The fish were transported in a plastic container containing the farm pond water to the Fish Farm of the Department of Fisheries, Modibbo Adama University, Yola. The fish were acclimatised for five days. Then the fish were randomly grouped into five treatments of 10 fish in three replicates. A mini flow-through system, with a 0.25m depth, 0.55m diameter, and 50 litres water holding capacity circular plastic bowls (filled with water up to 40 litres), was used for the feeding experiments. Water was supplied from a 30,000-litre overhead tank. The experimental fish were fed at 3% body weight at 6:00 hour and 18:00 hour daily for 6 weeks. Growth performance and feed utilisation in terms of percentage (%) weight gain, specific growth rate, feed conversion rate, and protein efficiency ratio were determined as described in Idowu (2019).

Mean weight gain (MWG):

$$\text{MWG (g/fish)} = \frac{\text{Wf-Wi}}{\text{n}} \quad (1)$$

Where Wf = final weight of fish (at the end of the experiment)

Wi = initial weight of fish (at the beginning of the experiment)

n = number of weeks

Specific growth rate (SGR):

$$\text{SGR (\%/day)} = \frac{\ln \text{Wf} - \ln \text{Wi}}{\text{t}} \times 100 \quad (2)$$

Where $ln\ Wf$ = natural logarithm of fish final weight

$ln\ Wi$ = natural logarithm of fish initial weight

t = experimental period in days

Condition factor (k):

$$k = \frac{100W}{l^3} \quad (3)$$

Where W = weight of fish
l = length of fish

Survival (S %):

$$S\ (\%) = \frac{Nf}{Ni} \times 100 \quad (4)$$

Where Nf = Number of calculated fish stocked (at the beginning of the experiment)
Ni = Number of fish alive (at the end of the experiment)

Feed conversion rate (FCR):

$$FCR = \frac{Feed\ intake\ or\ diet\ fed\ (g)}{Fish\ weight\ gained\ (g)} \quad (5)$$

Protein efficiency rate (PER):

$$PER = \frac{Fish\ weight\ gain\ (g)}{Protein\ fed\ (g\ of\ protein\ in\ 100g\ of\ diet/fish)} \quad (6)$$

Haematological Analysis

Three fish from each group were randomly selected for blood sampling at the start and end of the feeding trials for haematological analysis (Wijendra and Pathiratne, 2007). Blood samples were collected into 5ml EDTA sampling bottles from the caudal vein of fish using a sterile 2.5ml plastic syringe and needle and transported to the Haematology Unit, Medical Laboratory of Adamawa State Specialist Hospital, Jimeta-Yola, for haematological analysis. The haematological analysis of the blood samples was carried out using Sysmex Automated Haematology Systems (K×21N Model). Haematological indices, including white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin, mean cell haemoglobin concentration (MCHC), platelets (PLT), lymphocytes, and neutrophils were determined.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) tests to compare among treatment means, and the Duncan Multiple Range Test was used to separate the means.

RESULTS

The water quality parameters (Table 2): temperature was between 27°C and 28.6°C, dissolved oxygen between 4.58mg/l and 4.66mg/l, and pH, between 6.72 and 7.50. The experimental diet recorded a crude protein value of 40.36%, while the crude lipid and crude fibre were 7.01% and 5.43%, respectively (Table 3). There were significant differences ($p<0.05$) in the growth parameters of *C. gariepinus* fed the experimental diets (Table 4). Diet ALD2 recorded the highest mean weight gain (14.73±2.58 g/fish). Diet ALD3 recorded a survival of 80%, while other diets recorded 100%. The highest protein efficiency ratio

(0.58±0.09) was recorded for ALD2. The haematological indices of *C. gariepinus* fed diets incorporated with *Aloe vera* leaves-paste (Table 5) showed varying significant differences ($p<0.05$) in the indices evaluated. The highest white blood cells (270.70×10^3/µl) and red blood cells (2.08×10^6/µl) were recorded for ALD2. The platelets values ranged from 108×10^3/µl to 141×10^3/µl. Lymphocytes recorded values with no significant difference ($p>0.05$) from the initial value of 98.50% at the start of the feeding trials. Neutrophil was reported to be highest (2.40%) in CTRL and lowest (1.60%) in ALD2.

Table 2. Water Quality Parameters

Parameters	Minimum	Maximum
Temperature (°C)	27.00	28.60
Dissolved oxygen (mg/L)	4.58	4.66
pH	6.72	7.50
Ammonia (mg/L)	0.027	0.045

Table 3. Proximate Composition of the Experimental Basal Feed

Composition	Composition (% dry matter)
Crude Protein	40.36
Crude lipid	7.01
Crude Fibre	5.43
Ash	6.52
Nitrogen free-extract	32.18
Dry Matter	91.50

Table 4. Growth Parameters and Feed Utilization Indices of *Clarias gariepinus* Fed *Aloe vera* Leaves-Paste Diets

Parameters	CTRL	ALD1	ALD2	ALD3	ALD4
MIW (g/fish)	7.88 ± 0.37^{c}	8.11 ± 0.52^{b}	8.21 ± 0.91^{a}	8.08 ± 0.07^{b}	7.92 ± 0.19^{c}
MFW (g/fish)	11.61 ± 0.43^{c}	18.85 ± 2.56^{b}	22.93 ± 3.49^{a}	18.74 ± 0.02^{b}	11.19 ± 0.15^{c}
MWG (g/fish)	3.73 ± 0.06^{c}	10.74 ± 3.28^{b}	14.73 ± 2.58^{a}	9.16 ± 2.03^{b}	3.27 ± 0.34^{c}
SGR (%/day)	1.29 ± 0.50^{c}	1.99 ± 0.49^{ab}	2.44 ± 0.08^{a}	1.81 ± 0.27^{b}	0.82 ± 0.08^{c}
Survival (%)	100^{a}	100^{a}	100^{a}	80^{b}	100^{a}
CF (K)	0.39 ± 0.08^{bc}	0.49 ± 0.05^{a}	0.55 ± 0.03^{a}	0.43 ± 0.06^{b}	0.46 ± 0.05^{ab}
FCR	0.31 ± 0.04^{b}	0.15 ± 0.04^{c}	0.21 ± 0.00^{c}	0.23 ± 0.06^{bc}	0.52 ± 0.05^{a}
PER	0.29 ± 0.01^{c}	0.47 ± 0.07^{b}	0.58 ± 0.09^{a}	0.43 ± 0.06^{b}	0.28 ± 0.00^{c}

Values in the same row with different superscripts are significantly different ($p<0.05$)

CTRL- Control, ALD – *Aloe vera* leaves-paste diet, MIW – Mean initial weight, MFW- Mean final weight, MWG- Mean weight gain, SGR- Specific growth rate, CF- Condition factor, FCR- feed conversion ratio, PER- protein efficiency ratio

Table 5. Haematological Indices of *Clarias gariepinus* Fed A*loe vera* Leaves-Paste Diets

Parameters	INI	CTRL	ALD1	ALD2	ALD3	ALD4
WBC ($\times10^3\mu l^{-1}$)	212.60±1.02^{c}	223.10±2.21^{c}	261.40±1.56^{ab}	270.70±1.01^{a}	254.20±3.00^{b}	263.10±2.05^{ab}
RBC ($\times10^6\mu l^{-1}$)	1.63±0.04^{c}	1.66±0.21^{c}	1.99±0.05^{ab}	2.08±.024^{a}	1.95±0.30^{b}	2.00±0.12^{a}
HGB(g/dl)	7.10±0.30^{c}	7.20±1.0^{c}	9.40±0.20^{a}	9.70±1.20^{a}	8.80±0.30^{b}	9.30±1.00^{a}
HCT (%)	20.20±0.20^{c}	21.30±0.23^{c}	28.30±0.30^{a}	28.50±0.35^{a}	26.60±0.30^{b}	26.70±0.33^{b}
MCV (fl)	123.90±7.13^{d}	128.30±8.12^{cd}	142.20±11.20^{a}	137.00±8.00^{a}	136.40±13.20^{b}	133.50±9.12^{bc}
MCH (pg)	43.60±3.45^{b}	43.40±2.00^{b}	47.20±1.60^{a}	46.60±1.81^{a}	45.10±3.45^{ab}	46.50±5.12^{a}
MCHC(g/dl)	35.10±1.52^{a}	33.80±2.04^{ab}	33.20±1.50^{b}	34.00±2.70^{a}	33.10±2.27^{b}	34.80±1.98^{a}
PLT ($\times10^3\ \mu l^{-1}$)	82.00±2.06^{d}	118.00±1.96^{b}	93.00±1.82^{c}	141.00±0.69^{a}	108.00±2.10^{b}	131.00±1.71^{a}
LYM (%)	98.50±1.76^{a}	97.60±2.13^{a}	98.30±2.22^{a}	98.40±3.01^{a}	98.10±1.22^{a}	98.30±2.00^{a}
NEUT (%)	1.50±0.11^{b}	2.40±0.02^{a}	1.70±0.10^{b}	1.60±0.04^{b}	1.90±0.05^{ab}	1.70±0.06^{b}

Values in the same row with different superscript are significantly different (p<0.05)

CTRL-Control, ALD- *Aloe vera* leaf diet, WBC - White blood cells, RBC - Red blood cells, HGB – Haemoglobin, HCT – Haematocrit, MCV – Mean cell volume, MCH –Mean cell haemoglobin, MCHC – Mean cell haemoglobin concentration, PLT – Platelets, LYM – Lymphocytes, NEUT – Neutrophil

DISCUSSION

In this present study, the water quality parameters of the feeding trial groups were within the recommended limits for *C. gariepinus* (Boyd *et al*., 2020). Excellent water environment reduces stress and supports fish wellbeing (Idowu *et al*., 2017). This indicated that the water environment in the feeding trial groups provided appropriate living conditions for the experimental fish.

The crude protein content of the experimental diets corresponded with the dietary protein requirement for catfish (Nwanna *et al*., 2014). Protein is considered the most essential nutrient for fish growth and maintenance of their physiological functions (Sogbesan, 2014). The crude fat content in this study was below the 15% limit suggested for catfish (Robinson and Li, 2015). Too much dietary lipid, 15% or more in catfish diets, lowers feed efficiency and retards fish weight gain (Robinson and Li, 2015). The crude fibre content of the diets was within the preferable range, 3-6%, for catfish for better feed digestibility, protein utilisation and growth performance (Sogbesan, 2014).

Weight gain and specific growth rate are usually considered primary factors of the productivity of a diet and reliable indications of the marketability of fish feed (Sogbesan, 2014). The weight gains reported in all the treatments in this study compared to the control were indications that incorporation of *Aloe vera* leaves-paste has a considerable influence on the feed utilisation and growth performance of the experimental fish, with the diet ALD2 having the best growth performance indices. Gabriel *et al*. (2019) reported that an inclusion of *A. vera* powder at 0.5% and 2.0% per kg diet significantly enhanced feed utilisation and growth performances in *Oreochromis niloticus*. Similar improved feed utilisation and growth performances were reported in cultured *C. gariepinus* fingerlings fed dietary *Aloe barbadensis* leaves (Adegbesan *et al*., 2018). Also, a comparable performance was reported in *Cyprinus carpio* juveniles when fed diets supplemented with ethanolic *A. vera* extracts at 0.5% and 2.5% (Mahdavi *et al*., 2013). On the other hand, the same inclusion levels (0.1% and 1.0% *A. vera*/kg diet) that were concluded to have increased growth in *Oncorhynchus mykiss* (Heidarieh *et al*., 2013) have been reported to have no effect on the growth of the same fish species (Farahi *et al*., 2012; Golestan *et al*., 2015). However, the varying feed utilisation and growth performances reported in this study might be associated with antinutritional factors present in the *A. vera* leaves, their levels of inclusion, and synergetic relationship

with other ingredients in the experimental diets (Radha and Laxmipriya, 2014; Idowu, 2019).

The haematological parameters reported in this present study revealed improved values for *C. gariepinus* fingerlings fed diets incorporated with *A. vera* leaves-paste. Fish fed 0.5%, 1.0%, and 1.5% *A. vera* leaves-paste diets had better haematological profiles, with 1.0% *Aloe vera* leaves-paste diet as the optimum. This corresponded with the results obtained by Adegbesan *et al*. (2018), which revealed that haematological parameters of *C. gariepinus* fingerlings were enhanced after being fed varying levels of *A. barbadensis* leaves incorporated diet for 12 weeks. The increased red blood cell and white blood cell counts reported in this study for fish fed a 1.0% *Aloe vera* leaves-paste diet might be an indication that incorporation of *A. vera* leaves-paste at 1.0% in fish diets can stimulate formation of red blood cells, hence, increasing oxygen-carrying capacity in fish blood, and as well stimulate white blood cell formation, hence, strengthening fish innate defence mechanism against physiological stress (Radha and Laxmipriya 2014; Idowu, 2019). This present study reported higher neutrophil values in the fish fed *Aloe vera* leaves-paste diets. Alishahi and Abdy (2013) reported that 0.5% dietary *A. vera* increased serum bactericidal activity and antibody levels in *C. carpio* infected with *Aeromonas hydrophila*. According to Zodape (2010), in their research on *Labeo rohita*, they reported that *A. vera* can effectively enhance biological functions in fish under culture, and they attributed this to bioactive compounds in *A. vera*.

CONCLUSION

This present study indicated that *A. vera* leaves-paste diets can enhance fish growth performance and immunological profiles. The study further indicated that *C. gariepinus* can be fed diets incorporated with 1.0% *Aloe vera* leaves-paste for better growth and feed utilisation performances, survival, and immunological profiles. However, further studies into the synergetic effects of other feed ingredients and *A. vera* with well-defined constituents are required for standardisation and better comparison.

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