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Growth performance, haematology and histopathology of African catfish (*Clarias gariepinus*) fed varying levels of *Aloe barbadensis* leaves

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Abstract

One hundred and twenty *Clarias gariepinus* fingerlings (2.33 ± 0.07 g) were fed with 40% crude protein diets containing three concentrations of *Aloe barbadensis* leaves-paste: ABL1, 1%; ABL2, 2%; ABL3, 3%, and control, 0% *ad libitum* twice daily for 12 weeks. Mean weight gain and percentage weight gain increased (P < 0.05) as concentration of *A. barbadensis* increased. Survival rate decreased as concentration of paste increased. Differences (P < 0.05) seen in packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC), thus highest in ABL3: PCV (36.67 ± 0.89%), Hb (12.37 ± 0.37 g dl⁻¹) and RBC (3.47 ± 0.08×10⁶ L⁻¹) and lowest in control: PCV (22.0 ± 0.58%), Hb (7.37 ± 0.20 g dl⁻¹) and RBC (2.07 ± 0.06 ×10⁶ L⁻¹). Liver histology of control fish was normal, while fatty degenerations were seen in the treated fish. The histology of fish kidney was normal in all treatments. The study concluded that 1% *A. barbadensis* leaves-paste could effectively improve growth performance, nutrient utilization and survival of cultured *C. gariepinus*.

Keywords: Growth performance; Aloe barbadensis leaves-paste; dietary supplement; Clarias gariepinus

1 | INTRODUCTION

According to Food and Agriculture Organization (FAO) there is a gradual increase in aquaculture production (inland and marine) from about 55.7 million tons in 2009 to 167.2 million tons in 2014 (FAO 2016). Aquaculture as is an emerging industrial sector requires continued research with scientific, technical developments and innovations (Alicia *et al.* 2005; Mohsin *et al.* 2012; Galib *et al.* 2013) in different aspects of production including the search for natural alternative growth promoters to be used as feed supplements. Several studies had been carried out in developing new dietary supplementation strategies in which various health and growth promoting compounds like probiotics, prebiotics, synbiotics and phytobiotics have been used (Denev 2008). To develop alternative practices for growth promotion and disease management in aquaculture, attention has also been focused in identifying novel drugs, especially from plant sources. These drugs may be delivered to the cultivable organisms through feed supplementation. Several herbs have been confirmed as growth promoters in aquatic animals (Rao *et al.* 2006; Immanuel *et al.* 2007). Phytobiotics are plantderived, natural compounds and medicinal herbs embedded into diets to enhance animal productivity. These include Aloe vera (*Aloe barbadensis*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) widely used as growth promoters in different species of fish (Adedeji *et al.* 2008).

Aloe vera is a tropical or sub-tropical plant with turgid lance-shaped green leaves with jagged edges and sharp points (Qiao et al. 2013). It is a perennial plant belonging to the Liliaceae or Aloeaceae family and a succulent cactus-like plant, which grows in hot and dry climates (Choi and Sung 2003). Aloe vera is made up of a colourless liguid product, called gel consisting primarily water and polysaccharides, and a yellow latex representing 20 – 30% by weight of whole leaf with bitter taste, has aloe-emodin, aloin and barbaloin as its active ingredients (Plaskett 1998; Vogler and Ernst 1999). 75 compounds are present in aloe vera leaf, and each with several remedial properties. These include lignin, saponins, anthraquinones, minerals, vitamins, 28 amino acids, enzymes and sugars (monosaccharides and polysaccharides) (Banwankar et al. 2014). The polysaccharides include pectin, cellulose, hemi cellulose, glucomannan, accemannan and mannose derivatives (Lee et al. 2001).

Aloe vera does not affect food taste or appearance, so it is a safe, natural alternative to synthetic preservatives (Chandrakesan *et al.* 2009). *A. barbadensis*, is a growth promoting plant and also enhances immune responses in fish (Alishahi *et al.* 2010). A study examining aloe vera leaves at two concentrations (5 and 10%) in cockerels' diets reported that 5% aloe vera leaves improved body weights and feed efficiency of birds (Odo *et al.* 2010). Mmereole (2011) also reported that 1% aloe vera leaves in broiler diet enhanced body weight gain in the birds. It is noteworthy that *A. barbadensis* leaves-paste has not been used as a phytobiotics in fish species. Hence, this study aims at determining the growth promoting effect, haematology and histopathology of *Clarias gariepinus* fed different concentrations of *A. barbadensis* leaves-paste.

2 | METHODOLOGY

The research work was done at the fish farm (hatchery unit) of the Department of Aquaculture and Fisheries Management, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria from April 2015 to September 2015. The feeding trial was conducted in twelve (12) rectangular plastic tanks each with a capacity of 60 L of fresh water and each tank was two-third filled (40 L).

2.1 | Experimental fish

African mud catfish (*C. gariepinus*) fingerlings of mean weight 2.33 g were used as the test fish species in this study. A total of one hundred and twenty (120) fingerlings were purchased at Motherhood Fish Farm, Abeokuta,

Ogun State, Nigeria. The fish were randomly (completely randomized design) allotted into four (4) treatments in the plastic tanks at a stocking rate of ten fingerlings per tank in triplicates.

2.2 | Experimental diets

A ration of 40% crude protein (CP) containing fishmeal (72% CP), soybean meal (42% CP), groundnut cake (45% CP), using yellow maize (10% CP) as the energy source and fixed ingredients including vitamin premix (1%), lysine (0.5%), methionine (0.5%), di calcium phosphate (0.5%); salt (0.5%) and vegetable oil (4.0%). Afterwards, fresh leaves of aloe vera (A. barbadensis) were obtained from a herbarium in Sagamu, Ogun State and authenticated by a botanist. The leaves were thoroughly washed with distilled water and weighed on an electronic scale (Mettler Toledo FB602, Jenway UK), cut into pieces with a knife and blended in electric blender (Binatone, BLG 555, China) with water added at a ratio of 1:1 as described by Muhammad-Jameel et al. (2014) to give A. barbadensis leaves-paste. The proximate composition of A. barbadensis leaves-paste is shown in Table 1 following procedure described by AOAC (2011).

TABLE 1: Chemical composition of aloe vera leaves (Muhammad-Jameel *et al.* 2014).

Contents	Percentage (%)
Moisture	96.80 ± 0.45
Crude Protein	6.90 ± 0.06
Crude Fat	2.85 ± 0.05
Total ash	14.65±0.03

The aloe vera leaves-paste were added on top of the basal diet and thoroughly mixed to formulate four isonitrogeneous (40% crude protein) *A. barbadensis* leavespaste supplemented diets. Thus, the experimental diets are made up of three treatment diets containing different concentrations of *A. barbadensis* leaves-paste and the control as follows: Treatment 1 (Control), 0% *A. barbadensis* leaves-paste; Treatment 2 (ABL1), 1% *A. barbadensis* leaves-paste; Treatment 3 (ABL2), 2% *A. barbadensis* leaves-paste; and Treatment 4 (ABL4), 3% *A. barbadensis* leaves-paste.

The compounded feeds were pelletized (2 mm), sun dried, allowed to cool in an open air, packed and stored in an opaque nylon bag separately. The percentages of all the feed ingredients used in formulating the four experimental diets are listed in Table 2. The proximate analyses of the four experimental diets were carried out following AOAC (2011).

2.3 | Experimental procedure

The fish were acclimated to the experimental system for a

period of 14 days before the commencement of the feeding trial and were fed two times daily with a commercial diet (40% CP). The fish were weighed in batches; ten per treatments at the beginning of the experiment. Prior to the commencement of the experiment, all fish were starved for 24 hours to eliminate variation in weight due to residue food in the gut and at the same time to increase the appetite of the fish. Fish were fed with the diets at two feeding regimes, in the morning between 08:00 - 09:00 hr and in evening between 17:00 - 18:00 hr, ad libitum for (84 days) 12 weeks. The fish were weighed in each tank weekly using a sensitive electronic weighing scale (Mettler Toledo FB602, Jenway UK) to monitor the fish growth and ensure adequate feed consumption. Mortality was monitored daily. The proximate analyses of the fish carcasses were also carried out following AOAC (2011).

TABLE 2 Different feed ingredients and their percentages in the experimental diets

Ingredients	Control	ABL1	ABL2	ABL3
Fishmeal	31.2	31.2	31.2	31.2
Soybean meal	15.6	15.6	15.6	15.6
Groundnut cake	15.6	15.6	15.6	15.6
Yellow maize	30.5	30.5	28.75	27.75
Vitamin premix	1.0	1.0	1.0	1.0
Lysine	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vegetable oil	4.0	4.0	4.0	4.0
Methionine	0.5	0.5	0.5	0.5
DCP	0.5	0.5	0.5	0.5
A. barbadensis leaves (%)	0.0	1.0	2.0	3.0
Total	100	100	100	100

ABL, A. barbadensis leaves paste; DCP, Di calcium phosphate

2.4 | Water quality parameters

During study water temperature (°C) and dissolved oxygen (mg L⁻¹) were measured every week using a combined digital YSI dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio, USA); pH was also monitored weekly using pH meter (Mettler Toledo-320, Jenway UK). Status of different water parameters are shown in Figure 1.

2.5 | Haematological analysis

The blood samples were taken from the dorsal fin of the fish following Klontz and Smith (1986) and taken to the laboratory of Department of Veterinary Microbiology, College of Veterinary Medicine (COLVET), FUNAAB for haematological analysis. The samples were analysed according to methods adopted in fish haematology (Ivanova 1983; Haghighi 2010). The haematological parameters obtained include packed cell volume (PCV) or Haematocrit (HCT), Haemoglobin (Hb) and red blood cell (RBC). The absolute red blood cell indices (mean cell haemoglobin (MCH), mean cell volume (MCV), and mean cell haemoglobin concentrations (MCHC) were calculated. The white blood cell (WBC) and differential count (neutrophils and lymphocytes) were determined as described by Dacie and Lewis (2001).



FIGURE 1 Physicochemical parameters of water during the period of experiment

2.6 | Histopathology

The histopathological examinations were carried out on the liver and kidney of the fish at the Department of Veterinary Pathology, COLVET, FUNAAB. The organs were carefully removed from the body of the fish so as to avoid damage and preserved in 10% formalin solution. The fixed tissues were processed routinely for histological analysis as described by Samuelson (2007). The necrotized areas were then photographed and read accordingly to determine the histopathological effects of A. barbadensis leaves.

2.7 | Data analysis

Growth performance of fish was determined following the methods as illustrated by Agbebi et al. (2012) in term of final mean body weight, survival (%), specific growth rate, (SGR, % day⁻¹). The following growth parameters were calculated at the end of the study:

Percentage weight gain PWG (%)

- Final mean body weight $x \, 100$
- Initial mean body weight

Specific growth rate, SGR = $\frac{L_n W_2 - L_n W_1}{Time (days of experiment)}$ x 100

where,

 W_1 = initial weight gained; W_2 = final weight gained; L_n = natural logarithm

Survival rate

 $= \frac{No of fish remaining at the end of the experiment}{100} x 100$ No of fish at the beginning of the experiment

Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) responses were

calculated using the following formulas:

Protein efficiency ratio (PER) Mean weight gain Average crude protein fed

where,

Mean weight gain (MWG) = Final weight (g) of fish – Initial weight (g) of fish

=

Feed Conversion Ratio (FCR) = $\frac{Dry Weight of feed fed (g)}{Fish Weight gained}$

$$ANPU = \frac{Net \ Protein \ in \ Carcass}{Protein \ fed} x \ 100$$

$$Protein \ intake = \frac{Total \ feed \ consumed \ x \ Crude \ protein \ in \ feed}{100}$$

All data obtained were subjected to one way analysis of variance (ANOVA). Data were checked for normality before ANOVA and found suitable for the parametric test. Duncan Multiple Range Test (Duncan 1955) was used for comparison among diets means at an α significance level of 0.05. All the tests were carried out using statistical software SAS (Version 15.0).

3 | RESULTS

3.1 | Proximate composition of experimental diets

The crude protein contents of the diet ranged between 39.98 and 40.01%, crude fibre 3.04 and 3.42%, ether extract 4.70 and 5.42% and nitrogen free extract 35.77 and 38.60 (Table 3).

TABLE 3 Proximate composition of the experimental diets

Percentage of dry weight				
Control	ABL1	ABL2	ABL3	
10.50	10.98	9.86	9.56	
40.01	40.00	40.04	39.98	
3.10	3.12	3.04	3.42	
5.20	4.45	3.95	3.74	
5.42	5.20	4.98	4.70	
35.77	36.25	38.13	38.60	
	Percenta Control 10.50 40.01 3.10 5.20 5.42 35.77	Percentage of dry Control ABL1 10.50 10.98 40.01 40.00 3.10 3.12 5.20 4.45 5.42 5.20 36.25 36.25	Percentage of dry weight Control ABL1 ABL2 10.50 10.98 9.86 40.01 40.00 40.04 3.10 3.12 3.04 5.20 4.45 3.95 5.42 5.20 4.98 35.77 36.25 38.13	

ABL = Aloe barbadensis leaves

3.2 | Carcass compositions of experimental fish

The initial and final carcass compositions of the fish fed with varying levels of *A. barbadensis* leaves and the control are presented in Table 4. Moisture content ($12.40 \pm 0.08\%$) and crude protein content ($49.84 \pm 0.21\%$) was highest in ABL1.

3.3 | Growth Performance and nutrient utilization

The growth performance and nutrient utilization of *C. gariepinus* fed *A. barbadensis* leaves paste at three varying levels of dietary supplementation are shown in Table

5. Final weight (20.28 \pm 0.72 g), weight gain (17.95 \pm 0.78 g) and percentage weight gain (772.2 \pm 54.94 %) was highest in ABL3 (Table 5).

TABLE 4 Initial and final carcass compositions of fishes

Compose Percentage of dry weight (Mean + SD)					
compo-	Fercer				
nents	Initial	Control	ABLI	ABLZ	ABL3
Moisture	11.54	11.84 ±	12.40 ±	11.61 ±	12.26 ±
		0.23 ^b	0.08 ^a	0.08 ^b	0.02 ^b
Crude pro-	43.50	47.37 ±	49.84 ±	49.50 ±	49.50 ±
tein		0.55 ^d	0.21 ^c	0.02 ^c	0.39 ^c
Fibre		1.23 ±	1.28 ±	1.27 ±	1.48 ±
	0.90	0.02 ^c	0.10 ^c	0.04 ^c	0.02 ^b
Ash	0.98	4.18 ±	3.41 ±	3.47 ±	3.84 ±
		0.31 ^a	0.01 ^b	0.01 ^b	0.11 ^b
Ether ex-	8.50	12.44 ±	11.93 ±	10.69 ±	12.33 ±
tract		0.08 ^a	0.23 ^a	0.02 ^b	0.08 ^a
Nitrogen	34.58	22.95 ±	21.14 ±	23.47 ±	20.81 ±
free extract		0.09 ^a	0.28 ^b	0.04 ^a	0.43 ^c

Means along the same row with same letter are not significantly different (p > 0.05)

TABLE 5 Growth performances and nutrient utilization of*Clarias gariepinus*fed*Aloe barbadensis*leaves-supplemented diets

Daramatora	Values (Mean ± SD)				
Parameters	Control	ABL1	ABL2	ABL3	
Initial weight	2.30 ±	2.30 ±	2.33 ±	2.33 ±	
(g)	0.06 ^a	0.06 ^a	0.07 ^a	0.07 ^a	
Final weight (g)	14.22 ±	16.39 ±	18.79 ±	20.28 ±	
	1.12 ^c	0.33 ^{cb}	0.57 ^b	0.72 ^ª	
Weight gain (g)	11.92 ±	14.09 ±	16.46 ±	17.95 ±	
	1.16 ^c	0.34 ^c	0.52 ^b	0.78 ^ª	
Percentage	518.3 ±	613.7 ±	705.6 ±	772.2 ±	
weight gain (%)	10.21 ^c	22.28 ^b	13.93 ^ª	54.94 ^ª	
Feed intake (g)	19.17 ±	22.08 ±	25.66 ±	27.56 ±	
	2.29 ^c	0.51 ^{cb}	1.25 ^b	1.01 ^ª	
Feed conver-	1.40 ±	1.57 ±	1.56 ±	1.54 ±	
sion ratio	0.07 ^b	0.01 ^a	0.05 ^ª	0.02 ^ª	
Protein intake	7.67 ±	9.05 ±	10.27 ±	11.02 ±	
	0.92 ^c	0.15 ^b	0.50 ^b	0.41 ^ª	
Specific growth	2.31 ±	2.36 ±	2.48 ±	2.57 ±	
rate (%/day)	0.19 ^b	0.02 ^b	0.02 ^a	0.07 ^a	
Protein effi-	1.80 ±	1.56 ±	1.61 ±	1.63 ±	
ciency ratio	0.09 ^ª	0.03 ^b	0.05 ^b	0.02 ^b	
ANPU (%)	50.29 ±	70.08 ±	63.61 ±	57.12 ±	
	1.38 ^d	2.51 ^ª	4.23 ^b	2.19 ^c	
Survival rate	86.67 ±	83.33 ±	73.33 ±	76.67 ±	
(%)	8.82 ^ª	3.33 ^b	3.33 [°]	6.67 [°]	

Means along the same row with same letter are not significantly different (p > 0.05); ANPU = Apparent net protein utilization

3.4 | Haematological indices of experimental fish

PCV was significantly higher in fish fed the diets supplemented groups than the control (Table 6). Other haematological indices of fish fed varying levels of A. barbadensis leaves and the control are also shown in Table 6.

3.5 | Histopathology of experimental fish

Figure 2 represents fragment of the liver tissue showing regular hepatocyte in cords and plates with normal portal tract and central vein. Vacuolar degenerations were observed in the fish livers from the A. barbadensis leavespaste (Figures 3-5). There was moderate and diffuse vacuolar degeneration of hepatocyte, mild fibrous connective tissues proliferation (necrosis) was also seen (Figure 3); Figure 4 reveals moderate vacuolar degeneration of hepatocytes with fibrous connective tissues proliferation. There was marked and diffuse vacuolar fatty degeneration of the hepatocytes, moderate infiltration of the hepatocyte cytoplasm by lobules of matured adipocytes (Figure 5).

TABLE 6 Haematological parameters of Clarias gariepinus
 fed Aloe barbadensis leaves-supplemented diets

Onumentere	Values (Mean ± SD)				
Fulumeters	Control	ABL1	ABL2	ABL3	
PCV (%)	22.00 ±	29.00 ±	25.67 ±	36.67 ±	
	0.58 ^d	3.18 ^b	3.18 ^c	0.89 ^ª	
Hb (g dl ⁻¹)	7.37 ±	9.67 ±	8.57 ±	12.17 ±	
	0.20 ^d	0.20 ^b	1.01 ^c	0.32 ^ª	
RBC (×10 ⁶ L ⁻¹)	2.07 ± 0.06 ^d	2.73 ± 0.06 ^b	2.42 ± 0.30 ^c	3.44 ± 0.07 ^a	
MCH (pg)	35.60 ±	35.35 ±	35.47 ±	35.32 ±	
	0.07	0.01	0.19	0.18	
MCHC (g dl ^{-1})	33.33 ±	33.27 ±	33.61 ±	33.28 ±	
	0.00	0.04	0.22	0.08	
MCV (fl)	10.71 ±	10.63 ±	10.56 ±	10.62 ±	
	0.03	0.02	0.02	0.03	
WBC (×10 ³)	11.20 ±	12.30 ±	11.87 ±	12.67 ±	
	0.52 ^b	0.64 ^ª	0.32 ^b	0.32 ^ª	
Neutrophils (%)	40.00 ±	34.67 ±	33.00 ±	38.67 ±	
	1.73 ^ª	1.45 [°]	4.62 ^c	1.45 ^b	
Lymphocytes (%)	56.67 ±	62.67 ±	64.67 ±	58.00 ±	
	1.45 ^b	0.88 ^b	4.91ª	1.16 ^c	
Eosinophils (%)	3.00 ±	2.67 ±	2.67 ±	3.00 ±	
	0.00 ^a	0.33 ^b	0.33 ^b	0.00 ^a	
Monocytes (%)	0.67 ± 0.33	0.67±0.33	0.00±0.00	0.67 ± 0.33	
Basophils (%)	0.00 ±	0.00 ±	0.00 ±	0.00 ±	
	0.00	0.00	0.00	0.00	

Means along the same row with same letter are not significantly different (p > 0.05



FIGURE 2 Histological section FIGURE 3 Histological section of liver of fish fed with control of liver of fish fed 1% A. bardiet

badensis leaves



FIGURE 4 Histological section of liver of fish fed 2% A. barbadensis leaves

FIGURE 5 Histological sections of liver of fish fed 3% A. barbadensis leaves



FIGURE 6 Histological section of kidney of fish fed control diet

FIGURE 7 Histological section of kidney of fish fed 1% A. barbadensis leaves



FIGURE 8 Histological section FIGURE 9 Histological section of kidney of fish fed 2% A. barbadensis leaves

of kidney of fish fed 3% A. barbadensis leaves

The histological sections of the kidney of fish fed varying levels of *A. barbadensis* leaves paste and the control are shown in Figures 6 – 9. Figure 6 reveals kidney tissues with regular epithelial cells and no physical damage done to the tissues. Figure 7 shows fragment of kidney tissue showing moderate diffuse vacuolar degeneration of the epithelia cells of the tubules. Necrosis was revealed in fish fed 2% (ABL2) *A. barbadensis* leaves (Figure 8). Figure 8 reveals that the kidney with moderate diffuse vacuolar degeneration of the epithelia cells of the epithelia cells of the tubules of the tubules with mild fatty change. Figure 9 indicates fragment of kidney tissue with mild diffuse vacuolar degeneration of renal tubules.

4 | DISCUSSION

There was a general increase in weight gain in the course of the experiment with the highest growth performance observed in fish fed 3% *A. barbadensis* leaves (ABL3). This aligned with the work of Bello *et al.* (2012) who recorded similar increase in weight gain of fish when fed diets supplemented with walnut leaf and onion bulb residues. The increase in the growth rate of *C. gariepinus* in the first few weeks of culture in the study may be due to initial starvation of the fish which made them more metabolically active, which is similar to Obasa and Faturoti (2001) observation in juvenile *Heterotis niloticus*. They recorded an increase in growth of the fish as they were subjected to delay in feed distribution.

The superior performances of fish fed the supplemented diets in PWG, SGR and ANPU over control diet could be due to the presence of growth promoters, stimulants or constituents in *A. barbadensis* leaves (glucomannans, acemannan). This is in accord with the result of Muhammed-Jameel *et al.* (2014) who found that inclusion of aloe vera leaves up to 2% in the diet showed better growth performance of Fayoumi chicks. This was corroborated by Heidahieh *et al.* (2013) who demonstrated that high levels (2%) of aloe vera had a positive effect on growth performance in rainbow trout.

However, the feed intake increased as the concentrations levels of *A. barbadensis* leaves increased. The increased feed intake observed in this experiment in diet supplemented groups could be attributed to change in feed taste and stimulated appetite (Windisch *et al.* 2008). This result is in agreement with Darabighane *et al.* (2012) who reported increased feed intake in the supplemented groups which were treated by 2% aloe vera gel dissolved in water. The increased FCR recorded in fish fed supplemented diets than the control is similar to the report of Bello *et al.* (2012) who revealed that inclusion of 1.5% walnut leaf increased FCR in the supplemented groups than the control. This was also corroborated by Abd-El-Rhman (2009) which showed that the addition of propolis

- ethanolic extract and crude propolis increased the FCR, FER and PER in the supplemented groups when compared with the control. No significant differences (p > 0.05) in FCR among all dietary ginger powder treatments are available (Zomrawi et al. 2011) which conforms to the result obtained in this study. According to De Silva (2001), feed conversion ratio is between 1.2-1.8 for fish fed carefully prepared diets, and the results from the present study falls within this range. The highest PER obtained in the control diet could be attributed to the absence of tannin in the feed. Also, Davies et al. (2006) observed that protein efficiency ratio, is a measure of how well the protein sources in a diet could provide the essential amino acid requirement of the fish fed the diet. The better SGR recorded in the supplemented diets is in correlation with the result of Abou-Zeid (2002) who showed that Allium sativum supplementation positively affected O. niloticus biomass and SGR.

The reduction in survival rate in fish fed the supplemented diets as recorded in this experiment could be a result of some phytochemicals inherent in these feeds. This result disagreed with the findings of Farah *et al.* (2012) who concluded that survival rate of fish was promoted in diets supplemented with *Mellisa officinalis* and aloe vera.

Haematological parameters are routinely used for the evaluation of physiological environment and husbandry stressors in fishes (Ranza-faival *et al.* 2000). The changes in the blood characteristics of *C. gariepinus* caused by stress due to exposure to environmental pollutant, diseases or by pathogens have been studied by many researchers especially in capture fisheries (e.g. Ezeri 2001; Gabriel *et al.* 2001). Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle 1998).

The PCV of 22 to 37% observed in this study is within the range of 20 to 50% reported by Pietse *et al.* (1981). The present study indicates that *C. gariepinus* fed *A. barbadensis* leaves paste showed significant (p < 0.05) increase in haematocrit (PCV), haemoglobin, RBC, WBC, except for WBC of 2% inclusion of ABL in comparison to the control. This was in agreement with the work of Haghigi and Rohani (2013) who observed similar increase in the haematological parameters in rainbow trout fed ginger powder. These were also similar to the findings of Farah *et al.* (2012) who reported significant enhancement (higher values) of WBC and PCV in diet supplemented with *M. officinalis* and aloe vera. De Pedro *et al.* (2005) indicated that total and differential leukocyte counts are important indices of non-specific defense activities in fish.

The increased WBC and lymphocytes as the level of inclu-

sion in the blood of fish fed A. barbadensis leaves (Table 8) may be attributed to increased production of leucocytes in the hematopoietic tissue of the liver. This agreed with the work of Schaperclaus et al. (1991) who also recorded similar increase in WBC and lymphocytes in the blood of C. carpio fed aloe vera. The WBC and lymphocytes are the defense cells of the body. Douglas and Jane (2010) demonstrated that the amount of WBC and lymphocytes in the blood has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or circulating system (Oyawoye and Ogunkunle 1998). The range of RBC $(2.07 \times 10^{6} \text{ to } 3.47 \times 10^{6} \text{ mm}^{3})$ recorded in this study is comparable with 2.24×10^6 to 2.49×10⁶ mm³ reported by Sotolu and Faturoti (2009) in a study on growth performance and haematology of C. gariepinus fed varying inclusion of Leucaena laucocephala leaf meal. Reduction in the RBCs was observed in fish fed ABL2 and could be ascribed to the higher concentration of anti -metabolites especially tannin. This is in agreement with the work of Dienye and Olumuji (2014) who observed similar decrease in RBC in fish fed higher level of M. oleifera leaf meal.

There were no significant difference in MCH, MCHC and MCV observed in fish fed varying levels of *A. barbadensis* leaves paste and the control. This is in correlation with Haghigi and Rohani (2013) who observed no significant difference in MCV value in ginger powder fed diet and control.

The histopathology of the liver of fish fed with control diet showed that the liver was normal with mild fatty change. The mild fatty change might be attributed to the high fat content of catfishes. The histological sections of fish fed ABL1 and ABL2 were also normal with necrosis; however, the liver of fish fed ABL3 revealed marked and diffuse vacuolar fatty degeneration of the hepatocytes. This is in agreement with the report of Rapatsa and Moyo (2014) who observed vacuolation in fish livers fed M. oleifera diet treatment and the control. Also, histological sections of the liver of fish fed ABL3 indicated fish health was compromised at the highest dose. This is corroborated with the finding of Ashade et al. (2014) who revealed that liver of fish fed 30% untreated ginger peel had a severe fatty change. The presence of diffuse vacuolar degeneration of hepatocytes in fish fed varying levels of A. barbadensis leaves and may be as a result of excessive work required by the fish's liver to get rid of the plant toxicant from its body during the process of detoxification. This is corroborated by the work of Bamidele et al. (2015) who revealed similar effect on the fish liver.

It is noteworthy that vacuolar degeneration is a morphopathological alteration of the gastro-intestinal tract, and it may be associated with toxins and/or infection, which causes significant loss of water and potassium. Steatosis (lipid accumulation in the liver cells) could be present when there is excessive fat to be metabolized, or the lipid function of the liver cells are impaired due to hypoxia, toxic damage or certain infectious diseases (Szende and Suba 1999). Both vacuolar degeneration and fatty degeneration are reversible injuries, and cells can recover their normal functions (homeostasis) when the stress is removed (Szende and Suba 1999). Nonetheless, the recovery of cells will depend on the severity and duration of exposure to stressors.

The absence of visible changes in the histological sections of the kidney of fish with varying dietary doses of A. barbadensis leaves could be as a result of tolerability of the dietary supplements to the fish kidneys. This was in agreement with the observation of Bamidele et al. (2015) who found similar result on the kidney of C. gariepinus fed M. oleifera seed meal. This is corroborated by Daudu (2012) who observed no renal damage done in the broiler chickens fed ginger by product meal. Necrosis was observed in fish fed ABL2 which is in line with the report of Agbebi et al. (2013) who observed tubular degeneration and necrosis of *C. gariepinus* fed 10 g ginger powder. This portends the fact that lower concentration of A. barbadensis leaves as the 1% dietary supplementation in the present study is tolerable by fish metabolic organs such as the liver and kidney.

5 | CONCLUSION

The present study showed that dietary supplementation of *A. barbadensis* leaves paste is encouraged to improve the growth performance; nutrient utilization and health of catfish (*C. gariepinus*) fingerlings, due to the growth promoting and immunostimulation properties. However, the inclusion of *A. barbadensis* leaves beyond 2% in the diet of fish will cause histopathological disturbances on the liver, *A. barbadensis* leaves dietary supplementation are regarded to be tolerable to the fish kidney. Hence, for better growth performance, nutrient utilization and fish survival, it is inferred that 1% *A. barbadensis* leaves paste could be used as a supplement in the diet of fish. However, more research should be carried out on the uses of *A. barbadensis* in order to reduce the anti-nutritional factors and better utilize the plant.

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SIA primary data collection, statistical analysis and manuscript (MS) writing; SOO primary supervisor of the study; IA cosupervisor of the study.