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Effects of Dietary *Aloe barbadensis* (Aloeaceae) Leaves on the Intestinal Microbes of African Catfish (*Clarias Gariepinus* Burchell 1822)

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Abstract

Effects of *Aloe barbadensis* leaves-paste were examined in *Clarias gariepinus* fingerlings. Fish were fed diet supplemented with the leaves-paste to determine the effects of the leaves on the intestinal microbes of *C. gariepinus* fingerlings. Its effects on some growth parameters were also assessed. Experiment was conducted in 40-litre freshwater-filled plastic tanks. 120 *C. gariepinus* fingerlings (2.33±0.07g) were fed with 40% crude protein diets containing three concentrations of *A. 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 the concentration of *A. barbadensis* increased. Apparent net protein utilization was highest in ABL1 and decreased as inclusion level increased among fish fed the leaves. Survival rate decreased as concentration of paste increased. There were no significant differences ($p>0.05$) in the total bacterial counts (TBC) in *A. barbadensis* leaves-paste supplemented diets and the control having the highest TBC ($23.67 \pm 0.88 \times 10^5$ CFU/ml). No growth of total fungal counts (TFC) was observed in ABL1 and there was a reduction in TFC as the concentration increased in the other supplemented diets, and the control having the highest TFC ($7.67 \pm 0.44 \times 10^5$CFU/ml) The study concluded that 1% *A. barbadensis* leaves-paste could effectively improve the growth performance, nutrient utilization, survival and thus reducing the microbial load of cultured *C. gariepinus*.

Keywords: Growth Performance; *Aloe barbadensis* leaves-Paste; Dietary Supplement; *Clarias Gariepinus*

Introduction

Aquaculture is one of the most rapid growing food producing sectors in the world [1]. Aquaculture as is an emerging industrial sector requires continued research with scientific, technical developments and innovations [2] in different aspects of production including the search for natural alternative growth promoters to be used as feed supplements. The use of antibiotics and chemicals in production is often costly and unacceptable since they lead to antibiotics and chemical resistance, this uncontrolled and repeated uses of antibiotics to treat bacterial infections have in some cases led to the development of antibiotic resistant pathogens [3,4]. Thus, the use of immunostimulants in feeds is considered as a modern and promising alternative to antibiotics and vaccines often employed as prophylactic measures in intensive aquaculture [5]. Immunostimulants have the competence to promote the nonspecific resistance of fish before infection of pathogens. In recent decades, many substances have been shown to enhance the nonspecific immunity of fish and the route of their administration has differential effects on the immune system.

Current advancement in immuno-nutrition studies have shown that some nutrients are linked to the immunological status of fish [6]. This has drawn the attention of fish nutritionists to the immune-protection of fish besides the growth; sustainable aquaculture depends on perfect balance between growth and health condition of fish. In aquaculture, one of the most important methods of increasing the defense mechanism and disease management in fish is through prophylactic administration of immunostimulants in the fish [7]. Also, it has been described by various authors that the use of immunostimulants seems to be an alternative to control fish diseases and enhancements of growth [8,9]. The active principles of certain herbs have growth advancing ability and perform as appetizers, trigger the immune system, perform as wide spectrum antimicrobial and include anti-stress properties which will be of massive function in fish and shrimp cultivation.

Aloe vera (*A. barbadensis*) is a tropical or sub-tropical plant with turgid lance-shaped green leaves with jagged edges and sharp points [10]. 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 [11]. Aloe vera is made up of a colourless liquid product, called gel consisting primarily water and polysaccharides, and a yellow latex representing 20-30% by weight of whole leaf with bitter taste. Seventy-five potentially active compounds like enzymes, minerals, sugars, saponins etc are present in *aloe vera* [12]. Active compounds present in *A. barbadensis* include the following: polysaccharides, accemannans, anthraquinones, lectins, salicylic acid, urea, nitrogen, amino acids, lipids, sterols, tannins, phenol and enzymes [13,14]. Accemannan is the main functional component of aloe vera and is made up of long chain acetylated mannose [15]. *A. barbadensis*, a natural immunostimulant and growth promoting plant enhance some of the specific and non-specific immune responses by increasing lysozyme activity, serum bactericidal power and the total protein and immunoglobulin levels in fish [16]. Hence, this study aims at determining the growth promoting effect, nutrient utilization and anti-microbial action of *C. gariepinus* fed different concentrations of *A. barbadensis* leaves-paste.

Materials and Methods

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. The feeding trial was conducted in twelve (12) rectangular plastic tanks each with a capacity of 60 litres of fresh water and each tank was two-third filled (40 litres).

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.

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. [17] to give *A. barbadensis* leaves-paste.

The aloe vera leaves-paste prepared were added on top of the basal diet and thoroughly mixed using a mixer to formulate four iso-nitrogeneous (40% crude protein) *A. barbadensis* leaves-paste 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 listed below:

Treatment 1 (Control)-0% *A. barbadensis* leaves-paste
Treatment 2 (ABL1)-1% *A. barbadensis* leaves-paste
Treatment 3 (ABL2)-2% *A. barbadensis* leaves-paste
Treatment 4 (ABL4)-3% *A. barbadensis* leaves-paste

The compounded feeds were pelletized (2mm) using the pelletizing machine from University fish farm, sun dried, allowed to cool in an open air, packed and stored in an opaque nylon bag according to the treatments. The percentage of all the feed ingredients used in formulating the four experimental diets is listed in Table 1. The proximate analyses of the four experimental diets were carried out following the procedure described by AOAC [18].

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	1	1	1
Lysine	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vegetable Oil	4	4	4	4
Methionine	0.5	0.5	0.5	0.5
DCP	0.5	0.5	0.5	0.5
A. barbadensis leaves	0	1	2	3
TOTAL	100	100	100	100
Moisture	10.5	10.98	9.86	9.56
Crude protein	40.01	40	40.04	39.98
Fibre content	3.1	3.12	3.04	3.42
Ash	5.2	4.45	3.95	3.74
Ether extract	5.42	5.2	4.98	4.7
Nitrogen free extract	35.77	36.25	38.13	38.6

ABL = *A. barbadensis* leaves paste, DCP= Di calcium phosphate.
Table 1: Feed ingredients & Proximate Compositions of the Experimental Diets (% Dry weight).

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 08:00-09:00h and evening between 17:00-18:00h, *ad libitum* for (84 days) 12 weeks. The proximate analyses of the fish carcasses were also carried out following procedure as described by AOAC [18].

Monitoring of Fish Growth

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.

Growth parameters such as final mean body weight, survival (%), specific growth rate, (SGR, %/day), mean weight gain, percentage weight gain and feed intake were calculated according to the methods of Agbebi, et al*.* [19].

Nutrient utilization parameters such as feed conversion ratio, protein intake and apparent net protein utilization were also determined.

Determination of Intestinal Microflora of the Fish

Dissection of the midline in ventral surface of the fish was carried out to remove the gut proximal section. Hedges 2002 was used to estimating the precision of serial dilution of viable bacteria count and fungi count of the intestinal contents.

Various bacterial isolates were subjected to morphological and biochemical tests for their identification according to the methods of Buchanan, et al. [20]. The results were analyzed by cross reference to Bergey's Manual of Systematic Bacteriology [20]. Fungal isolates were subjected to morphological characteristics according to Barnett, et al. [21] and identified according to Campbell, et al. [22].

Statistical Analysis

All data obtained were subjected to one way analysis of variance (ANOVA). Duncan Multiple Range Test was used for comparison among diets means at a significance level of 0.05 ($p < 0.05$) [23]. The computations were subjected to SAS statistical software version 15.

Results

Proximate Composition of Experimental Diets

Proximate compositions of the four diets formulated and prepared for the feeding trial are presented in Table 1. The crude protein contents of the diet ranged between 39.98 and 40.09%, crude fibre 3.04 and 3.42%, ether extract 4.50 and 5.42% and nitrogen free extract 35.77 and 38.60.

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 is presented in Table 2. There was no significant difference ($p > 0.05$) in the moisture content of fish fed high doses (2% and 3%) of *A. barbadensis* leaves-paste supplemented diets and the control, while the fish fed 1% *A. barbadensis* leaves diet recorded the highest moisture content Table 5. There were also no significant differences ($p > 0.05$) in the crude protein and ash contents of the carcass of fish fed *A. barbadensis* leaves.

Proximate components (%)	Initial	Control (0%)	ABL1 (1%)	ABL2 (2%)	ABL3 (3%)
Moisture	11.54	11.84±0.23^{b}	12.40±0.08^{a}	11.61±0.08^{b}	12.26±0.02^{b}
Crude protein	43.5	47.37±0.55^{d}	49.84±0.21^{c}	49.50±0.02^{c}	49.50±0.39^{c}
Fibre content	**0.9**	1.23±0.02^{c}	1.28±0.10^{c}	1.27±0.04^{c}	1.48±0.02^{b}
Ash	0.98	4.18±0.31^{a}	3.41±0.01^{b}	3.47±0.01^{b}	3.84±0.11^{b}
Ether extract	**8.5**	12.44±0.08^{a}	11.93±0.23^{a}	10.69±0.02^{b}	12.33±0.08^{a}
Nitrogen free extract	34.58	22.95±0.09^{a}	21.14±0.28^{b}	23.47±0.04^{a}	20.813±0.43^{c}

Means along the same row with same letter are not significantly different (p >0.05).
Table 2: Proximate compositions of the fish (% Dry weight) (Mean ± SEM).

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 is shown in Table 3. There was significant difference (p < 0.05) in the final mean weights gain of the fish at the end of the experiment. The highest final mean weight gain recorded in fish fed 1% *A. barbadensis* leaves paste was significantly higher (p<0.05) than the lowest in fish fed the control diet. With respect to the values obtained in weight gain (WG) and percentage weight gain (PWG), WG and PWG increased as the level of inclusion of *A. barbadensis* leaves-paste increased which were significantly different (p>0.05) from the control. However, the SGR and feed conversion ratio (FCR) of fish fed *A. barbadensis* leaves paste were not statistically different (p>0.05) from that fed the control diet. The results showed that feed intake increased as the concentrations increased in fish fed the *A. barbadensis* leaves paste diets with highest value in ABL3 compared with the control. The result of this experiment showed that FCR was best in fish fed control diet which was significantly lower than in fish fed the diets supplemented groups and there were no significant differences (p>0.05) in the FCR among the fish fed the diets supplemented groups.

Nutrients were better utilized among the diets supplemented group at different significant levels (p<0.05) with highest value of ANPU in ABL1 when compared to all other treatments including the control. There was no significant difference (p>0.05) in values obtained for protein efficiency ratio (PER) in fish fed the diets supplemented groups. Fish fed the control diet recorded the highest PER value. The survival rate was highest in fish fed the control diet followed by fish fed the ABL1 and there were no statistically different (p>0.05) in values obtained between ABL2 and ABL3. Percentage survival decreases as the concentrations increases in fish fed *A. barbadensis* leaves paste supplemented diets.

Parameters	Control (0%)	ABL1 (1%)	ABL2 (2%)	ABL3 (3%)
Initial weight (g)	2.30±0.06^{a}	2.30±0.06^{a}	2.33±0.07^{a}	2.33±0.07^{a}
Final weight (g)	14.22±1.12^{c}	16.39±0.33^{cb}	18.79±0.57^{b}	20.28±0.72^{a}
Weight gain (g)	11.92±1.16^{c}	14.09±0.34^{c}	16.46±0.52^{b}	17.95±0.78^{a}
Percentage Weight gain (%)	518.3±10.21^{c}	613.7±22.28^{b}	705.6±13.93^{a}	772.2±54.94^{a}
Feed intake (g)	19.17±2.29^{c}	22.08±0.51^{cb}	25.66±1.25^{b}	27.56±1.01^{a}
Feed conversion ratio	1.40±0.07^{b}	1.57±0.01^{a}	1.56±0.05^{a}	1.54±0.02^{a}
Protein intake	7.67±0.92^{c}	9.05±0.15^{b}	10.27±0.50^{b}	11.02±0.41^{a}
Specific growth rate (%/day)	2.31±0.19^{b}	2.36±0.02^{b}	2.48±0.02^{a}	2.57±0.07^{a}
Protein efficiency ratio	1.80±0.09^{a}	1.56±0.03^{b}	1.61±0.05^{b}	1.63±0.02^{b}
ANPU (%)	50.29±1.38^{d}	70.08±2.51^{a}	63.61±4.23^{b}	57.12±2.19^{c}
Survival rate (%)	86.67±8.82^{a}	83.33±3.33^{b}	73.33±3.33^{c}	76.67±6.67^{c}

Means along the same row with same letter are not significantly different (p>0.05).
ANPU= Apparent net protein utilization
Intestinal Microflora of the Fish
Table 3: Growth performance and nutrient utilization of *Clarias gariepinus* fed *Aloe barbadensis* leaves-supplemented diets (Mean ± SEM).

Microbial Count of Fish

The total bacteria count and fungal counts in the intestine of fish fed *A. barbadensis* leaves diets and the control are presented in Table 4. The microbial population of fish fed diet containing varying levels of *A. barbadensis* leaves were greatly reduced compared to the control (p<0.05). There were no significant differences (p>0.05) in the total bacterial counts (TBC) in *A. barbadensis* leaves-paste supplemented diets and the control having the highest TBC. No growth of total fungal counts (TFC) was observed in ABL1 and there was a reduction in TFC as the concentration increased in the other supplemented diets, and the control having the highest TFC.

Parameters	Control (0%)	ABL l (1%)	ABL 2 (2%)	ABL3 (3%)
Total Bacteria Count (CFU/ml) x10^5	23.67±0.88[a]	16.83±1.59[b]	17.67±1.17[b]	16.67±4.26[b]
Total Fungal Count (CFU/ml) x10^5	7.67±0.44[a]	0.00±0.00	3.85±1.80[b]	3.1±0.73[c]

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

Table 4: Microbial load in the intestine of *C. gariepinus* fed *A. barbadensis* leaves (Mean ± SEM).

Morphological Characteristics of Bacteria Colonies Isolates

The cultural and colonial morphology of bacteria found in the intestine of fish fed varying level of *A. barbadensis* leaves and the control are shown in Table 5.

Isolates	Color	Shape/Margin	Arrangement	Surface appearance	Elevation	Texture	Opacity
A1	Creamy	Irregular	Cocci in chain	Smooth and Glistening	Raised	Dry	Translucent
A2	Creamy	Irregular	Cocci in chain	Smooth and Glistening	Raised	Mucoid	Translucent
B1	Creamy	Irregular	Cocci in pair or more	Smooth and Glistening	Raised	Mucoid and Moist	Translucent
B2	Creamy	Irregular	Cocci in pair	Smooth and Glistening	Raised	Mucoid	Translucent
C1	White	Entire	Cocci	Glistening	Raised	Dry	Translucent
C2	Yellow	Entire	Cocci in cluster	Smooth	Raised	Moist	Translucent
D1	White	Entire	Cocci in pair	Glistening	Raised	Dry	Translucent
D2	Yellow	Entire	Cocci in cluster	Smooth	Raised	Moist	Translucent

A = Control
B = ABL1
C = ABL2
D = ABL3

Table 5: Morphological characters of bacteria colonies isolates of *C. gariepinus* fed *A. barbadensis* leaves diets.

Biochemical Characterization of the Bacteria Isolate

Table 6 shows the biochemical test of the bacteria isolated from the intestine of the fish showing the occurrence of the bacteria in the fish intestine. *Streptococcus agalactiae*, *Enterococcus faecialis* and *Staphylococcus aureus* were the bacteria found in the fish intestine. *E. faecialis* occurred mostly in the fish intestine, however *S. agalactiae* and *S. aureus* are the least occurred as shown in Figure 1.

ISOLATE	GRAM	SPORES	MOTILITY	CATALASE	STARCH HYDROLYSIS	GELATIN LIQUEFACTION	OXIDASE	MANNITOL	SUCROSE	LACTOSE	GLUCOSE	SUSPECTED ORGANISM
A1	+	-	-	-	-	-	-	-	+	-	+	*Streptococcus agalactiae*
A2	+	-	-	-	-	-	-	-	+	-	+	*S. agalactiae*
B1	+	-	-	-	+	-	-	+	-	+	+	*Enterococcus faecialis*

B2	+	-	-	-	+	-	-	+	-	+	+	*E. faecialis*
C1	+	-	-	-	+	-	-	+	-	+	+	*E. faecialis*
C2	+	-	-	+	-	+	-	-	+	+	+	*Staphylococcus aureus*
D1	+	-	-	-	+	-	-	+	+	+	+	*E. faecialis*
D2	+	-	-	+	-	+	-	-	+	+	+	*S. aureus*

+: POSITIVE
-: NEGATIVE

Table 6: Biochemical test of the bacteria isolated from the intestine of C*. gariepinus* fed *A. barbadensis* leaves diets.

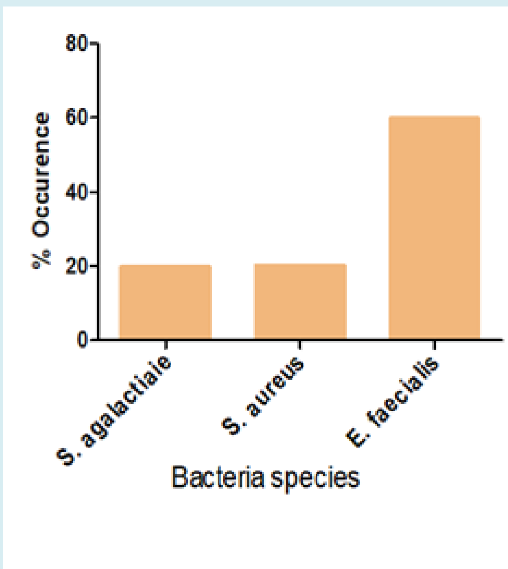

Figure1: Percentage occurrence of bacteria isolated from the intestine of fish fed *A. barbadensis* leaves.

Colonial Characteristics of Fungal Isolate

The morphological characters of fungal isolated from the intestine of *C. gariepinus* fed varying level of *A. barbadensis* leaves and the control are presented in Table 7. The prevalence of fungi in the intestine of the fish include; *Aspergillus niger, Mucor mucedo* and *Rhizopus Stolonifer.* The *A. niger* occurred mostly in the fish intestine followed by *M. mucedo* while *R. stolonifer* is the least occurred as indicated in Figure 2.

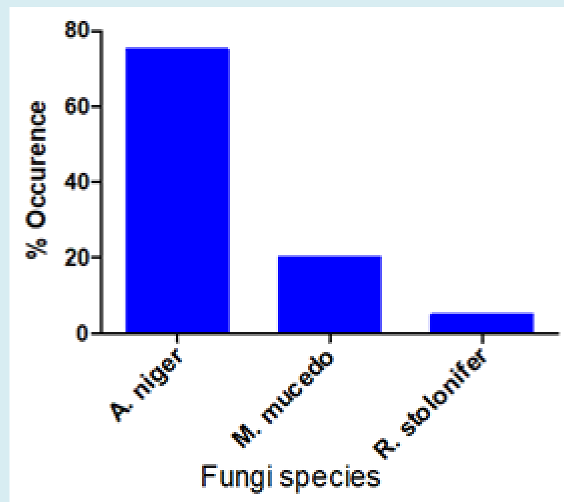

Figure 2: Percentage occurrence of fungi isolated from the intestine of fish fed *A. barbadensis* leaves.

Isolates	Color of spore	Appearance of mycelia	Type of Spores	Arrangement of spores	Shape of spore	Type of hyphae	Identified organism
A1	Yellow black	Fluffy	Sporangiospore	In masses	Globuse	Aseptate	*Mucor mucedo*
A2	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
B1	Nil	Nil	Nil	Nil	Nil	Nil	Nil
B2	Nil	Nil	Nil	Nil	Nil	Nil	Nil
C1	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
C2	Yellow black	Fluffy	sporangiospore	In masses	Globuse	Aseptate	*Mucor mucedo*
D_1	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
D2	Black	Caltony	sporangiosphore	In masses or singly	Irregularly round	Aseptate	*Rhizopus stolonifer*

Table 7: Fungal isolated from intestine of *C. gariepinus* fed *A. barbadensis* leaves diets.

Discussion

The values of the physico-chemical parameters observed in the experimental tanks during this study were within the range recommended for *C. gariepinus* [24,25]. The achievement of this was as a result of optimum water management practices.

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. This aligned with the work of Bello, et al. [26] 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, et al. [27] 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. [17] 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. [28] 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 as reported by Windisch, et al. [29]. This result is in agreement with Darabighane, et al. [30] 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. [26] who revealed that inclusion of 1.5% walnut leaf increased FCR in the supplemented groups than the control. This was also corroborated by Abdel-Rahman, et al. [31] 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. The findings of Zomrawi, et al. [32] who showed that were no significant differences ($p > 0.05$) in FCR among all dietary ginger powder treatments which conformed to the result obtained in this study. According to De Silva, et al. [33] 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. [34] 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, et al. [35] which showed that *Allium sativum* supplementation positively affected *O. niloticus* biomass and specific growth rate (SGR).

The reduction in survival rate in fish fed the supplemented diets as recorded in this experiment could be as a result of some phytochemicals inherent in them. This result of this present study disagreed with the findings of Farah, et al. [36] who concluded that survival rate of fish was promoted in diets supplemented with *Mellisa officinalis* and aloe vera.

The bacteria flora of the intestine of *C. gariepinus* consisted of *Streptococcus agalactiae, Staphylococcus aureus,* and *Enterococcus faecialis.* This could indicate that *A. barbadensis* leaves favours or promote the growth of gram positive bacteria in the intestine of *C. gariepinus.* This can be compared with the work of Olojo, et al. [37] who observed similar bacteria flora in the intestine of *C. gariepinus.* This is corroborated by Nwabueze, et al. [38] who observed similar bacteria flora from the epidermal mucus of *C. gariepinus* fed ginger powder. Pandy, et al. [39] also reported that all medicinal plants are able to stimulate only non-specific immune responses and suggested that vaccines might be a better way to prevent deadly diseases and as such, the medicinal plants could be used to replace the vaccine.

This microflora can also be advantageous in that they help in digestive processes of fish such as microbial breakdown of chitin, collagen, cellulose and they may also supply fatty acids and other vitamins to the host and hence promoting growth of the fish. These might have taken place in the fish fed the dietary supplements. The microflora also prevents colonization of the fish by other microbes that might otherwise be pathogenic. Tannins and saponins are present in *A. barbadensis* leaves, Tannins and saponins are responsible for antibacterial activity, and able to permeate cells without destroying cell morphology. Tannins inhibit microbial proliferation by denaturation of enzymes of involved in microbial metabolism [40]. This might have taken place in the intestine of fish fed the dietary supplement because majorly the bacteria flora in the intestine of *C. gariepinus* are mostly gram positive bacteria and thus these phytochemicals probably inhibited the growth of gram negative bacteria and promoted the growth of gram positive bacteria.

There were significant ($p<0.05$) a reductions in the total bacteria count of fish fed diets supplemented with varying levels of *A. barbadensis* leaves than the control. This is in agreement with the report of Bello, et al. [26] who observed that there was a decrease in values of the bacterial load of the supplemented groups (onion bulb and walnut leaves) as the level of inclusion (0.5%, 1.0% and 2.0%) increased and as the months increased. In this study, enterobacteriacea load in the intestine of *C. gariepinus* fed *A. barbadensis* leaves was lower than the control with significant decrease ($p<0.05$). This decrease in bacteria load in fish as observed in this study has been linked to the presence of antimicrobial properties in *A. barbadensis* leaves. This study suggests that is more effective as an antibacterial *A. barbadensis* leaves. Tannins also have shown potential antiviral, antibacterial properties [41].

The fungi flora of the intestine of *C. gariepinus* fed diets containing varying levels of *A. barbadensis* leaves and the control revealed the presence of *Aspergillus niger, Mucor mucedo* and *Rhizopus stolonifer. A. niger* and *M. mucedo* are associated to food spoilage. Their presence in the fish intestine could indicate that some of the experimental feeds might have been rancid and support the growth of these microbes.

Also, there were significant ($p < 0.05$) reductions in the total fungal count of fish fed diets supplemented with varying levels of *A. barbadensis* leaves than the control diet. Also, this study indicated that both *A. barbadensis* leaves could act effectively as an antifungal. This is in line with the work of Idris, et al. [42] who worked on the effect of different concentration of ginger on smoke-dried *C. gariepinus,* and found that ginger reduced the free fatty acid values, tri-methylamine values and reduced the fungi load of the processed fish [43-45].

Conclusion

The present study showed that dietary supplementation of *A. barbadensis* leaves paste is encouraged to improve the growth performance [46]; nutrient utilization and health of catfish (*C. gariepinus*) fingerlings, due to the growth promoting and immunostimulation properties. Based on this present study, *A. barbadensis* leaves paste has been shown to possess antibacterial and antifungal activities in the fish. 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 and also, *A. barbadensis* leaves paste could be used as an antibacterial and antifungal in cultured fish diets. 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 [47,48].

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