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Original Article

Effects of Dietary Supplementation of *Zingiber officinale* Root-Powder on Growth, Nutrient Utilization and Intestinal Microbes of African Mud Catfish (*Clarias gariepinus*) Fingerlings

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Abstract

Phytobiotics are valuable materials in promoting growth and reducing pathogenic diseases in cultured fish diets. To this effect, a 12 weeks experiment was conducted in a 40 litres fresh water filled rectangular plastic tanks to evaluating the growth, nutrient utilization potential and gasytointestinal microbes of *Clarias gariepinus* fed varying levels of *Zingiber officinale* root powder. 120 *C. gariepinus* fingerlings (2.33 ± 0.07 g average weight) were fed with 40 % crude protein diets containing three concentrations of *Z. officinale* roots-powder: ZOP1-1 %; ZOP2-2 %; ZOP3-3 %, and control-0 % *ad libitum* twice daily for 12 weeks. Significant differences ($p < 0.05$) occurred in the growth and nutrient utilization parameters except feed conversion ratio and specific growth rate.

Survival rate decreased as concentration of powder increased. The Total Bacterial Counts (TBC) and Total Fungal Counts (TFC) decreased as inclusion levels of the supplements increased which were different ($p < 0.05$) from the control. The study concluded that 1 % *Z. officinale* roots-powder dietary supplementation in cultured *C. gariepinus* could effectively promote growth and reducing the microbial load of the fish.

Keywords: *Clarias gariepinus*; Dietary supplement; Growth; Microbe; *Z. officinale* roots-powder

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Introduction

Aquaculture, one of the most important options in animal protein production. It however requires high quality feeds with high protein content and some complementary additives or dietary supplements to keep fish healthy and to enhanced growth [1]. Medicinal plant products (phytobiotics) have been shown to act as anti-stress, growth promoters, appetite stimulators, tonic and immunostimulants and to have antimicrobial characteristics in finfish and shell fish culture [2]. Phytobiotics are plant-derived, natural compounds embedded into diets which enhanced animal productivity and they include: *Aloe vera* (*Aloe barbadensis)*, garlic (*Allium sativum*) and ginger (*Zingiber officinale*) as growth promoters have been widely used in different species of fish [3]. All fish live in microbe-rich environment and are vulnerable to invasion by pathogenic and opportunistic micro-organisms. The environment of fish being aquatic is very challenging with fish in constant interaction with a wide range of pathogenic and non-pathogenic micro-organisms [4].

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. Rao et al. [5], reported that these drugs may be delivered to the cultivable organisms either through feed supplementation or oral delivery through diets mode.

(*Z. officinale*) belongs to the Zingiberaceae plant [6]. It is beneficial to growth and immune systems in aquatic animals [7]. The rhizome of *Z. officinale* has been reported to possess a broadspectrum of prophylactic and therapeutic activities [8]. It is important in fish diet in that it control infection [9]. The function the plant performed is linked to the active ingredients that are present in the plant. Themajor constituents in ginger rhizomes are carbohydrates (50-70 %), lipids (3-8 %), terpenes, and phenolic compounds [10]. Hence, this study aims at assessing the growth, nutrient utilization and anti-microbial potentials of cultured *Clarias gariepinus* [11] fed varying inclusion levels of *Z. officinale* root-powder.

Experimental system

The study was conducted 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 experiment was conducted in twelve (12) rectangular plastic tanks each (60 litres) and each tank was two third filled (40 litres).

Experimental fish

African mud catfish (*C. gariepinus*) fingerlings (2.33 g average weight) were used 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

Four iso-nitrogenous diets containing 40 % Crude Protein (CP) were formulated to containing three diets containing different concentrations of *Z. officinale* root-powder and the control as listed below:

- Treatment 1 (Control) - 0 %
- Treatment 2 (ZOP1) - 1 % *Z. officinale* root-powder
- Treatment 3 (ZOP2) - 2 % *Z. officinale* root-powder
- Treatment 4 (ZOP3) - 3 % *Z. officinale* root-powder

Diets formulation and preparation

A ration of 40 % Crude Protein (CP) containing fishmeal (72 % CP), groundnut cake (45 % CP), soybean meal (42 % CP) and 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 %). The weight of each ingredient was calculated using Pearson Square method using the stipulated crude protein requirement of the fish. All the four diets formulated were carefully prepared which involves measuring the ingredients, thoroughly mixing the ingredients, and pelletizing them as described by Fagbenro and Adebayo [12]. The ginger rhizome was prepared separately before incorporation into the basal diets.

Preparation and processing of Z. officinale root-powder

Fresh rhizomes of *Z. officinale* roots were bought from a local market in Abeokuta, Ogun state, Nigeria and were confirmed by a botanist. The plant was dried in the shade. The dried rhizomes were further grinded into powdered form using a household grinder and sieved using a household sieve as described by Haghighi and Rohani [13].

Incorporation of *Z. officinale* root-powder into the diets

The powdered *Z. officinale* root produced was mixed directly with the basal diet and added into the diets at concentration of 1 %, 2 % and 3 % of feed. Compounded feeds were pelletized (2 mm) using the pelletizing machine from University fish farm, sun dried and allowed to cool then packed and stored in an opaque nylon bag according to the treatments. The percentages of all the feed ingredients used are shown in table 1.

Proximate Analysis

The Proximate analysis of the four diets and the fish were done following procedure as described by AOAC [14]. The proximate composition of *Z. officinale* root-powder [15] is shown in table 2.

Experimental procedure

The fish were acclimated to the experimental system for a period of 14 days before starting the experiment and were fed two times daily with a commercial diet (40 % CP). The fish were weighed according to the set up per treatments at the beginning of the experiment.

Ingredients (%)	Control	ZOP1	ZOP2	ZOP3
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
Z. officinale root-powder	0.0	1.0	2.0	3.0
Total	100	100	100	100

Table 1: Feed ingredients and Proximate Compositions of the Experimental Diets (% Dry weight).

Parameter	Amount (%)
Crude protein	34.13
Crude fat	4.02
Ether extract	4.07
Ash content	7.64
Moisture content	13.75
Vitamin C	1.036

Table 2: Chemical composition of ginger root powder.

Fish feeding

All fish were starved for 24 hours to increase the appetite of the fish before starting the experiment. Fish were fed with the diets two times daily, in the morning between 09:00 - 10:00 h and evening between 16:30 - 17:30 h, *ad libitum* for 12 weeks.

Water quality management

Water temperature (°C) and dissolved oxygen (mg \ l) were measured every week using a combined digital YSI dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio); pH was monitored weekly using pH meter (Mettler Toledo-320, Jenway UK).

Monitoring of Fish Growth

Weighing of the fish were done in each tank weekly to monitor the fish growth and ensure adequate feed consumption using a sensitive electronic weighing scale (Mettler Toledo FB602, Jenway UK).

Determination of Gastrointestinal microbes of the Fish

Estimation of bacterial counts

The ventral surface of the fish was dissected to removing the gut proximal section. The fish guts (intestines) were gotten for estimation the precision of serial dilution of viable bacteria count and fungi counts as described by Hedges [16].

Microbial identification

The bacterial culture were isolated and were subjected to morphological and biochemical tests for their identification according to the

methods of Buchanan and Gibbson [17]. The results were analyzed by cross reference to Bergey's Manual of Systematic Bacteriology [17].

The fungal isolates were subjected to morphological characteristics according to Barnett and Hunter [18] and the isolated fungi were identified according to Campbell et al. [19].

Data Analysis

Analysis of fish growth performance

Growth performance of fish was done as illustrated by Agbebi et al. [20], in term of mean weight gain, percentage weight gain, survival (%), specific growth rate (SGR % / day). The growth parameters calculated at the end of the experiment were:

$$Percentage\ weight\ gain\ PWG(\%) = \frac{Final\ mean\ body\ weight}{Initial\ mean\ body\ weight} \times 100$$

$$Specific\ growth\ rate, SGR = \frac{L_n W_2 - L_n W_1}{Times\ (days\ of\ \exp eriment)} \times 100$$

Where,
W_1 = Initial weight gained
W_2 = Final weight gained
L_n = Natural logarithm

$$Survival\ rate = \frac{No\ of\ \ fish\ remaining\ at\ the\ end\ of\ the\ \exp eriment}{No\ of\ \ fish\ at\ the\ beginning\ of\ the\ \exp eriment} \times 100$$

Analysis of feed conversion and nutrient utilization

The nutrient utilization parameters such as the Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) responses were calculated and data were obtained from them.

$$\Pr otein\ Efficienvy\ Ration\ (PER) = \frac{Mean\ weight\ gain}{Average\ crude\ protein\ fed}$$

Where,

Mean Weight Gain (MWG) = Final weight (g) of fish - Initial weight (g) of fish

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

$$ANPU = \frac{Net\ \Pr otein\ in\ carcass}{\Pr otein\ \ fed} \times 100$$

$$\Pr otein\ Intake = \frac{Total\ \ feed\ consumed \times Crude\ protein\ in\ \ feed}{100}$$

Also, data was obtained from bacterial and fungal counts.

Statistical Analysis

The data obtained were subjected to one way Analysis of Variance (ANOVA). Means were separated using Duncan Multiple Range Test [21] at a significant level of 0.05. Computations were subjected to SAS statistical package version 15.0.

Results

Proximate composition of experimental diets

Proximate compositions of the four diets formulated and prepared for the feeding trial are presented in table 3. Crude protein of the experimental diets is 40 % among the four treatments, moisture content ranged between 9.56 and 11.0 %, ether extract ranged between 4.70 and 5.42 %, fibre contents ranged between 1.23 and 1.67 %.

Proximate components (%)	Control (0 %)	ZOP1 (1 %)	ZOP2 (2 %)	ZOP3 (3 %)
Moisture	10.50	10.98	9.86	9.56
Crude protein	40.01	40.00	40.04	39.98
Fibre content	3.10	3.12	3.04	3.42
Ash	5.20	4.45	3.95	3.74
Ether extract	5.42	5.20	4.98	4.70
Nitrogen free extract	35.77	36.25	38.13	38.60

Table 2: Proximate compositions of the experimental diets (% Dry weight).

Carcass compositions of experimental fish

The initial and final carcass compositions of the fish fed with varying levels of ginger root-powder and the control is presented in table 4. The crude protein content of the fish carcass ranged between 43 and 52.5 %.

Physicochemical parameters of the water

The physicochemical parameters of the water recorded during the experimental period are shown in table 5.

Growth Performance

The growth performance and nutrient utilization of *C. gariepinus* fed *Z. officinale* root-powder at three varying levels of dietary supplementation and the control is shown in table 6. There were differences in the mean Weight Gains (WG) among fish fed *Z. officinale* root-powder. There were significant differences in the mean weight gain among fish fed with the *Z. officinale* root-powder when compared to the fish fed with the control diets. The highest WG (18.0 g) and Percentage With Gain, PWG (838 %) was recorded in fish fed with 1 % *Z. officinale* root-powder while the lowest WG (11.9 g) and PWG (518 %) was recorded in fish fed with control diet. The highest Feed Conversion Ratio, FCR (1.57) was observed in fish fed with 1 % *Z. officinale* root-powder while the lowest FCR was recorded in fish fed with control diet. There were significant no differences in the protein efficiency ratio among fish fed with the *Z. officinale* root-powder.

Intestinal Microbes of the Fish

Microbial count of fish

The total bacteria count and fungal counts in the intestine of fish fed *Z. officinale* root-powder diets and the control are presented in table 7. The microbial population of fish fed diet containing varying levels of ginger roots powder was greatly reduced compared to the control ($p < 0.05$). A significant difference ($p < 0.05$) occurred in the total bacterial and a fungal count was recorded among the treatment groups with the control having the highest count. Fish fed 2 % *Z. officinale* root-powder diet recorded the least bacterial and fungal counts followed by 3 % *Z. officinale* root-powder fed diet.

Morphological characteristics of bacteria colonies isolates

The cultural and colonial morphology of bacteria found in the intestine of fish fed varying level of *Z. officinale* root-powder are shown in tables 8 and 9.

Proximate components (%)	Initial	Control (0 %)	ZOP1 (1 %)	ZOP2 (2 %)	ZOP3 (3 %)
Moisture	11.54	11.84 ± 0.23^{b}	11.92 ± 0.02^{a}	11.85 ± 0.03a	11.97 ± 0.04^{a}
Crude protein	43.50	47.37 ± 0.55^{c}	52.48 ± 0.65^{a}	50.83 ± 0.17^{b}	50.19 ± 0.38^{b}
Fibre content	0.90	1.23 ± 0.02^{c}	1.34 ± 0.05^{b}	1.60 ± 0.12^{a}	1.39 ± 0.04^{b}
Ash	0.98	4.18 ± 0.31a	3.83 ± 0.13^{b}	3.49 ± 0.01^{b}	3.57 ± 0.04^{b}
Ether extract	8.50	12.44 ± 0.08^{a}	9.85 ± 0.20^{b}	10.01 ± 0.28^{b}	9.58 ± 0.06^{b}
Nitrogen free extract	34.58	22.95 ± 0.09^{a}	19.58 ± 0.38^{c}	21.22 ± 0.60^{b}	23.30 ± 0.36^{a}

Table 4: Proximate compositions of the fish (% Dry weight) (Mean ± SEM).

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

Week	pH	Dissolved Oxygen (Mg / L)	Temperature (°C)
0	6.00	6.10	25.00
1	6.90	6.20	25.41
2	7.15	6.18	25.60
3	7.03	6.24	25.78
4	7.34	6.35	25.83
5	7.41	6.50	25.61
6	7.48	7.21	26.13
7	7.31	7.25	25.53
8	7.22	7.40	26.20
9	7.28	7.56	26.13
10	7.50	7.45	26.30
11	7.55	7.61	26.05
12	7.56	7.70	26.02

Table 5: Physicochemical Parameters of water during experimental period.

Biochemical characterization of the bacteria isolate

Table 10 shows the biochemical test of the bacteria isolated from the intestine of the fish fed *Z. officinale* root-powder and the control showing the occurrence of the bacteria in the fish intestine.

Colonial characteristics of fungal isolate

The morphological characters of fungal isolated from the intestine of C. gariepinus are presented in tables 11 and 12.

Discussion

The values of the physico-chemical parameters observed in the experimental tanks during this study were within the range recommended for *C. gariepinus* [22,23]. The achievement of this was as a result of optimum water management practices.

There was a general increase in weight gain with the highest growth performance observed in fish fed 1 % and 3 % *Z. officinale* root-powder. Fish fed 1 % *Z. officinale* root-powder diet recorded the highest and showing best growth responses in terms of Percentage Weight Gained (PWG), Specific Growth Rate (SGR) and Apparent Net Protein Utilization (ANPU). The fish fed the control diet recorded the lowest values of PWG and ANPU. This is in agreement with the work of Bello et al. [24], who recorded similar increase in weight gain of fish when fed diets supplemented with Walnut leaf and Onion bulb residues. There was a rapid growth rate of *C. gariepinus* in the first few weeks of culture in the study; this could probably due to initial starvation of the fish which made them more metabolically active. This is similar to report of Obasa and Faturoti [25] who recorded similar observation in the growth of juvenile *Heterotis niloticus*. They recorded an increase in growth of the fish as they were subjected to delay in feed distribution.

The remarkable performances of fish fed the supplemented diets in PWG, SGR and ANPU over control diet could be due to the presence of growth promoters: zingiberene, glycosides and terpenoids in 1 % *Z. officinale* root-powder. This is in agreement with the result of Onibi et al. [26], suggested that ginger and garlic supplements collectively or individually improved growth performance of broilers. This was corroborated by Haghighi and Rohani [13].

There was a reduction in the total feed intake at higher levels of (2 % and 3 %) of *Z. officinale* root-powder; this could probably due to lower palatability of the two diets as a result of the presence of tannin in the *Z. officinale* root-powder. The work of Fasasi et al. [12], stated that tannins interfere with digestion by displaying anti-trypsin and anti- amylase activity, forming complexes with vitamin B12 and interfering with the bioavailability of proteins. Also, authors such as Azaza et al. [27], are of opinion that the presence of 2.4 % tannin in faba beans (*Vicia faba* L. *var. minuta*) might be responsible for low palatability and consequently low feed intake in Nile tilapia. The increased FCR as revealed in fish fed *Z. officinale* root-powder and higher than the fish fed control diet is similar to the report of Bello et al. [24], who revealed that inclusion of 1.5 % walnut leaf increased FCR in the supplemented groups than the control. This was also corroborated by the work of Abd El - Rahman [28] which indicated that the inclusion of Propolis - ethanolic extract and crude propolis increased the FCR, FER and PER in the supplemented groups when compared with the control. Also the findings of Zomrawi et al. [29], who showed that were no significant differences ($p > 0.05$) in FCR among all dietary *Z. officinale* root-powder treatments which corroborated with the result obtained in this present study.

The better SGR recorded in the supplemented diets is in correlation with the result of Abou-Zeid [30] which showed that *Allium sativum* supplementation positively affected *O. niloticus* biomass and Specific Growth Rate (SGR).

There is a reduction in survival rate in fish fed the *Z. officinale* root-powder diets as seen in this experiment could be as a result of some phytochemicals present in the phytobiotic. Fish fed 2 % and 3 % *Z. officinale* root-powder recorded the highest mortality rate.

Parameters	Control (0 %)	ZOP1 (1 %)	ZOP2 (2 %)	ZOP3 (3 %)
Initial weight (g)	2.30 ± 0.06^{a}	2.33 ± 0.09^{a}	2.33 ± 0.03^{a}	2.33 ± 0.09^{a}
Final weight (g)	14.22 ± 1.12^{c}	20.27 ± 0.92^{a}	18.49 ± 1.09^{b}	20.14 ± 1.15^{a}
Weight gain (g)	11.92 ± 1.16^{c}	17.95 ± 0.90^{a}	16.15 ± 1.06^{b}	17.82 ± 1.07^{a}
Percentage weight gain (%)	518.3 ± 10.21^{c}	840.8 ± 7.70^{a}	691.4 ± 10.63^{cb}	762.3 ± 17.69^{b}
Feed intake (g / fish)	19.17 ± 2.29^{c}	27.11 ± 1.49^{a}	25.2 ± 1.65b	25.64 ± 1.96^{b}
Feed conversion ratio	1.40 ± 0.07^{c}	1.57 ± 0.04^{a}	1.56 ± 0.02^{a}	1.55 ± 0.02^{a}
Specific growth rate (% / day)	2.31 ± 0.19^{c}	2.57 ± 0.10^{a}	2.46 ± 0.06^{a}	2.56 ± 0.02^{a}
Protein efficiency ratio	1.80 ± 0.09^{c}	1.65 ± 0.04b	1.60 ± 0.02^{b}	1.61 ± 0.03^{b}
Apparent net protein utilization (%)	50.29 ± 1.38^{c}	83.04 ± 3.92^{a}	68.270 ± 5.12^{b}	65.60 ± 5.05^{b}
Survival rate (%)	86.67 ± 8.82^{c}	80.00 ± 5.77^{b}	63.33 ± 3.33c	63.33 ± 3.33^{c}

Table 6: Growth performance and nutrient utilization of *Clarias gariepinus* fed *Z. officinale* root-powder supplemented diets (Mean ± SEM).

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

Parameters	Control 0 %	ZOP1 1 %	ZOP2 2 %	ZOP3 3 %
Total Bacteria Count (CFU / ml) × 10^{5}	23.67 ± 0.88^{a}	17.33 ± 4.76^{b}	7.4 ± 5.31^{c}	8.84 ± 7.58^{c}
Total Fungal Count (CFU / ml) × 10^{5}	7.67 ± 0.44^{a}	2.83 ± 1.36^{d}	2.00 ± 0.00^{e}	2.50 ± 0.00^{e}

Table 7: Microbial load in the intestine of *C. gariepinus* fed *Z. officinale* root-powder (Mean ± SEM).

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

Isolates Details	A1	B1	C1	D1
Colour	Creamy	Dull Cream	Dull Cream	White
Shape / Margin	Irregular	Entire	Entire	Entire
Arrangement	Cocci in long chain	Baccilli	Bacilli	Cocci in pair
Surface Appearance	Smooth and Glistening	Smooth	Smooth	Smooth and Glistening
Elevation	Raised	Raised	Raised	Raised
Texture	Dry	Moist	Moist	Dry
Opacity	Translucent	Translucent	Translucent	Translucent

Table 8: Morphological characters of bacteria colonies isolates of *C. gariepinus* fed *Z. officinale* root-powder diets (1st replicate)

Key:

A = Treatment one (Control)
B = Treatment five (1 % of *Z. officinale* root-powder)
C= Treatment six (2 % of *Z. officinale* root-powder)
D = Treatment seven (3 % of *Z. officinale* root-powder)

Isolates Details	A2	B2	C2	D2
Colour	Creamy	Creamy	Creamy	White
Shape / Margin	Irregular	Entire	Entire	Entire
Arrangement	Cocci in chain	Baccilli	Baccilli	Cocci in pair
Surface Appearance	Smooth and Glisteningv	Smooth and Glistening	Smooth and Glistening	Smooth and Glistening
Elevation	Raised	Raised	Raised	Raised
Texture	Mucoid	Moist	Moist	Dry
Opacity	Translucent	Translucent	Translucent	Translucent

Table 9: Morphological characters of bacteria colonies isolates of *C. gariepinus* fed *Z. officinale* root-powder diets (2^{nd} replicate)

Isolate	Gram	Spores	Motility	Catalase	Starch hydrolysis	Gelatin liquefaction	Oxidase	Mannitol	Sucrose	Lactose	Glucose	Suspected organism
A1	+	-	-	-	-	-	-	-	+	-	+	Streptococcus agalactiae
A2	+	-	-	-	-	-	-	-	+	-	+	S. agalactiae
B1	+	+	+	+	+	+	+	+	-	-	+	Baccillus subtilis
B2	+	+	+	+	+	+	+	+	-	-	+	B. subtilis
C1	+	+	+	+	+	+	+	+	-	-	+	B. subtilis
C2	+	+	+	+	+	+	+	+	-	-	+	B. subtilis
D1	+	-	-	-	+	-	-	+	-	+	+	E. faecialis
D2	+	-	-	-	+	-	-	+	-	+	+	E. faecialis

Table 10: Biochemical test of the Bacteria isolates

Key: +: Positive,-: Negative

Treatment	Colour of spore	Appearance of mycelia	Type of spores	Arrangement of spores	Shape of spore	Type of hyphae	Identified organism
A_{11}	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*Aspergillus niger*
A_{12}	Yellow black	Fluffy	Sporangiospore	In masses	Globuse	Aseptate	*Mucor mucedo*
B_1	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
C_1	Yellow black	Fluffy	Sporangiospore	In masses	Globuse	aseptate	*Mucor mucedo*
D_1	Black	Grainy	Conidiosphore	In masses	Cylindrical	septate	*A. niger*

Table 11: Fugal Isolates from fish Intestine (1st replicate)

Treatment	Colour of spore	Appearance of mycelia	Type of spores	Arrangement of spores	Shape of spore	Type of hyphae	Identified organism
A_{21}	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
A_{22}	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
B_2	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
C_2	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*
D_2	Black	Grainy	Conidiosphore	In masses	Cylindrical	Septate	*A. niger*

Table 12: Fungal isolates from fish intestine (2nd replicate).

The findings of Ashade et al. [31], also revealed that the mortality rate of fish fed untreated ginger peel increased with respect to the different concentrations and the highest concentration having more mortality rate.

The bacteria flora of the intestine of *C. gariepinus* consisted of *Bacillus substilis, Streptococcus agalactiae* and *Enterococcus faecialis*. This could indicate that *Z. officinale* root-powder favours or promote the growth of gram positive bacteria in the intestine of *C. gariepinus*. This is in the agreement with the work of Olojo et al. [32] who observed similar bacteria flora in the intestine of *C. gariepinus*. This is corroborated by Nwabueze [11] who observed similar bacteria flora from the epidermal mucus of *C. gariepinus* fed ginger powder. Pandy et al. [33], also reported that all medicinal plants are able to stimulate only non-specific immune responses and suggested that the medicinal plants could be used to treat diseases. This gastrointestinal microbes could 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 [34]. These might have taken place in the fish fed the dietary supplement. The gastrointestinal microbes also prevent colonization of the fish by other microbes that might otherwise be pathogenic. *B. substilis* has been reported to promote performance and immune responses which might have taken place in fish fed ZOP1 and ZOP3. Tannins and alkaloid are present in ginger roots powder. 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 [35]. This might have occurred 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 differences ($p < 0.05$) in the reductions in the total bacteria count of fish fed varying levels of *Z. officinale* root-powder than the control. This is in agreement with the report of Bello et al. [24], 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. The enterobacteriaceae load in the intestine of *C. gariepinus* fed *Z. officinale* root-powder were lower than the control with significant decrease ($p < 0.05$) as recorded in this study. This decrease

in bacteria load in fish as observed in this study has been linked to the presence of antimicrobial properties in *Z. officinale* root-powder. This study suggests that *Z. officinale* root-powder is more effective as an antibacterial. Tannins also have shown potential antiviral, antibacterial properties [36].

The fungi flora of the intestine of *C. gariepinus* fed diets containing varying levels of *Z. officinale* root-powder revealed the presence of *Aspergillus niger* and *Mucor mucedo. 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 differences ($p < 0.05$) in the reductions in the total fungal count of fish fed diets supplemented with varying levels of *Z. officinale* root-powder than the control diet. Also, this study indicated that *Z. officinale* root-powder could act effectively as an antifungal. This is in line with the work of Idris et al. [37], which 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.

Conclusion

The results of this present study revealed that dietary supplementation of *Z. officinale* root-powder for *C. gariepinus* fingerlings are encouraged to improve the growth performance; nutrient utilization and gastrointestinal microbes of *C.* gariepinus fingerlings. This could be attributed to the growth promoting activity and immunostimulation properties of the dietary supplement. Inclusion level of 1 % *Z. officinale* root-powder in the diet of fish is best considered because of its good FCR, high ANPU, SGR and weight gain. Based on this present study, inclusion of 2 % *Z. officinale* root-powder is relatively considered to act as an antibacterial and antifungal in the fish and improving the gastrointestinal microbes of *C. gariepinus* fingerlings.

Authors' Contributions

The main author Mrs. Adegbesan S.I. of this work centers on carrying out this research in the field and manuscript preparation. The co-author, Prof. S.O. Obasa worked on manuscript proof reading and offering supervisory role for the conduct of this experiment. The area of gastro-intestinal microbes of this research was supervised by the second co-author, Prof. A.K. Akintokun a microbiologist. The third co-author, Dr. Abdulraheem I. assisted in the supervision of the aquaculture aspect of the research.

References

1. Sayed SH, Zakaria A, Mohamed GA, Mohamed KK (2011) Use of probiotics as growth promoter, anti-bacterial and their effects on the physiological parameters and immune response of *Oreochromis niloticus* Lin. fingerlings. Journal of the Arabian Aquaculture Society 6: 201-222.
2. Sivaram V, Babu MM, Citarasu T, Immanuel G, Murugadass S, et al. (2004) Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. Aquaculture 237: 9-20.
3. Adedeji OS, Farimi GO, Ameen SA, Olayemi JB (2006) Effects of bitter kola (*Garcinia kola*) as growth promoter in Broiler Chicks from day old to four weeks old. Journal of Animal and Veterinary Advances 5: 191-193.
4. Subramanian S, Mackinnon SL, Ross NW (2007) A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp Biochem Physiol B Biochem Mol Biol 148: 256-263.
5. Rao YV, Das J, Pradhana BK, Chakrabarthi R (2006) Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila.* Shell fish immunology 20: 263-273.
6. Akram M, Shah MI, Usmanghan K, Mohiuddinn E, Sami A (2011) *Zingiber officinale* Roscoe (A medicinal plant*).* Pakistan Journal of Nutrition 10: 399-400.
7. desouky H, Asely AE, Shaheem A, Abass A (2012) Effects of *Zingiber officinalis* and *Cyanodon dactylon* on the Growth Performance and Immune Parameters of *Macrobrachium rosenbergii*. World Journal of Fish and Marine Science 4: 301-307.
8. Ernst E (2000) Adverse effects of herbal drugs in dermatology. Br J Dermatol 143: 923-929.
9. Nya EJ, Austin B (2011) Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. Fish Shellfish Immunol 30: 845-850.
10. Grzanna R, Lindmark L, Frondoza CG (2005) Ginger--an herbal medicinal product with broad anti-inflammatory actions J Med Food 8: 125-132.
11. Nwabueze AA (2014) Antimicrobial action of epidermal mucus extract of *Clarias gariepinus* (Burchell, 1822) juveniles-fed ginger inclusion in diet. International Journal of Biology 6: 42-48.
12. Fasasi OS, Adeyemi IA, Fagbenro OA (2005) Proximate composition and multi-enzyme *in vitro* protein digestibility of maize-tilapia flour blend. Journal of Food Technology 3: 342-345.
13. Haghighi M, Rohani MS (2013) The effect of powered ginger (*Zingiber officinale)* on the haematological and immunological parameters of rainbow trout *Oncorrrhychus mykiss*. JMPHTR 1: 8-12.
14. AOAC (2011) Association of International Official Analytic Chemists, AOAC International, 18th edition, Arlington, Virginia.
15. Latona DF, Oyeleke GO, Olayiwola OA (2012) Chemical Analysis of Ginger Root, Journal of Applied Chemistry 1:47-49.
16. Hedges AJ (2002) Estimating the precision of serial dilutions and viable bacterial counts. International Journal of Food Microbiology 76: 207-214.
17. Buchanan RE, Gibbson ME (1994) Bergey's Manual of Systematic Bacteriology. The Williams and Wilkins Company, Baltimore, USA. Pg no: 510-593.
18. Barnett HL (1987) Illustrated General of Imperfect Fungi, (2rd Edn). Burgess Publishing Company, Minneapolis, USA. Pg no: 241.
19. Campbell CK, Johnson EM, Warnork DW (2013) Identification of Pathogenic Fungi, 2nd Edition. John Wiley & Sons, Inc Pg no: 1-350.
20. Agbebi OT, Lawal HB, Odebiyi VC (2012) Aflatoxin effect of moulded gel waste mixed with ginger and its histopathological study on *Clarias garepinus.* Global Journal of Science Frontier research*,* 12: 7-15.
21. Duncan DB (1955) Multiple range and multiple F tests. Biometrics 11: 1-42.
22. Adekoya BB, Olunuga OA, Ayansanwo TO, Omoyinmi GAK (2004) Manual of the second annual seminar and training workshop held at Ogun State Agricultural Development Programme, OGADEP, Olabisi Onabanjo way, Idi-Aba, Abeokuta. Publisher: Fisheries Society of Nigeria (Ogun State Chapter). Pg no: 52.
23. Omotayo AM Akegbejo-Samson Y, Olaoye OJ (2006) Fish Production, preservation, processing and storage, Training manual of the 2006 Joint training of fish farmers in Epe, Lagos State Agricultural media Resources and Extension Centre (AMREC), Federal University of Agriculture Abeokuta, Ogun State and BATN Foundation, Victoria Island, Lagos, Canada. Pg no: 19-20.

24. Bello OS, Emikpe BO, Olaifa FE (2012) The body weight changes and gut morphometry of *Clarias gariepinus* juveniles on feeds supplemented with Walnut (*Tetracarpidium conophorum*) Leaf and Onion (*Allium cepa*) Bub Residues. International Journal of Morphology 30: 253-251.

25. Obasa SO, Faturoti EO (2001) Growth response and serum component and yield of the African bony tongue (*Heterotis niloticus*) fed varying dietary crude protein level. ASSET Series A 1: 97-104.

26. Onibi GE, Adebisi O E, Fajemisin AN, Adetunji AV (2009) Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. African Journal of Agricultural Research 4: 511-517.

27. Azaza MS, Wassim K, Mensi F, Abdelmouleh A, Brini B, Kraiem MM (2009) Evaluation of faba beans (*Vicia faba* L. *var. minuta*) as a replacement for soybean meal in practical diets of juvenile Nile tilapia *Oreochromis niloticus.*Aquaculture 287: 174-179.

28. Abdel-Rahman AM (2009) Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, *Oreochromis niloticus*. Fish Shellfish Immunol 27: 454-459.

29. Zomrawi WB, Abdel Atti KHA, Mahala AG (2011) Effect of ginger root powder supplementation on broiler chicks performance, blood and serum constituents. Online Journal of Animal and Feed Research 1: 457-460.

30. Abou-zeid SM (2002) The effect of some medical plant on reproductive and productive performance of Nile tilapia, *Oreochronis niloticus*. Ph.D Thesis Cairo, Faculty of Agriculture, Cairo University, Giza, Egypt. Pg no: 212.

31. Ashade OO, Adelusi OE, Ligali NA (2014) Histopathological effects of untreated ginger peel (*Zingiber officinale*) fish meal on the intestine tissue profiling of African catfish (*Clarias gariepinus*) International Journal of Fisheries and Aquatic studies 2: 95-98.

32. Olojo EAA, Amusa NA, Osho A, Badejo VO (2010) Commensal bacterial flora of *Synodontis nigrita* and *Clarias gariepinus* from River Osun, Southwest Nigeria, Nigeria. Research Journal of Applied Sciences 5: 231-235.

33. Pandy G, Madhuri S, Mandloi AK (2012) Medicinal plants useful in fish diseases. International Research Journal of Pharmacy 12: 1-4.

34. Ringo E, Strom E, Tabachek JA (1995) Intestinal microflora of Salmonids: A review. Aquaculture Research 26: 1365-2109.

35. Awosika F (1991) Local medicinal plants and health of consumer. Clinical Pharmacognosis Herbal Medicine. 9: 28-29.

36. Akinyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K (2001) Antibacterial action of several tannins against *Staphylococcus aureus.* Journal of Antimicrobial Chemotherapy 48: 487-491.

37. Idris GL, Omojowo FS, Omojasola PF, Adetunju CO, Ngwu EO (2010) The effect of different concentrations of ginger on the quality of smoked dried catfish (*Clarias gariepinus*). Nature and Science 84: 59-63.

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