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 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 %. 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

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 made of (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 random 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

**Diet 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. Compound feeds were pelleted (2 mm) using the pelleting machine from University fish farm, sun dried and allowed to cool then packed and stored in an opaque nylon bag according to the set up per treatments at the beginning of the experiment.

**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.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Control</th>
<th>ZOP1</th>
<th>ZOP2</th>
<th>ZOP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>31.2</td>
<td>31.2</td>
<td>31.2</td>
<td>31.2</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>15.6</td>
<td>15.6</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>15.6</td>
<td>15.6</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>Yellow Maize</td>
<td>30.5</td>
<td>30.5</td>
<td>28.75</td>
<td>27.75</td>
</tr>
<tr>
<td>Vitamin Premix</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>DC P</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Z. officinale</em> root-powder</td>
<td>0.0</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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

**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⁻¹) 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 esti-

**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 Gibson [17]. The results were analyzed by
cross reference to Bergey’s Manual of Systematic Bacteriology [17].

The fungal isolates were subjected to morphological characteris-
tics 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 cal-
culated at the end of the experiment were:

\[
\text{Percentage weight gain (PWG)} = \left( \frac{W_f - W_i}{W_i} \right) \times 100
\]

\[
\text{Specific growth rate, SGR} = \left( \frac{\ln W_f - \ln W_i}{\text{times (days of experiment)}} \right) \times 100
\]

Where,

- \( W_i \) = Initial weight gained
- \( W_f \) = Final weight gained
- \( L_s \) = Natural logarithm

\[
\text{Survival rate} = \left( \frac{\text{No. of fish remaining at end of the experiment}}{\text{No. of fish at beginning of the experiment}} \right) \times 100
\]

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 Utili-
zation (NPU) responses were calculated and data were obtained from
them.

\[
\text{Protein Efficiency Ratio (PER)} = \left( \frac{\text{Mean weight gain}}{\text{Average crude protein fed}} \right)
\]

Where,

\[
\text{Mean Weight Gain (MWG)} = \text{Final weight (g) of fish - Initial weight (g) of fish}
\]

\[
\text{Feed Conversion Ratio (FCR)} = \left( \frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gained (g)}} \right)
\]

\[
\text{ANPU} = \left( \frac{\text{Net Protein in carcass}}{\text{Protein fed (g)}} \right) \times 100
\]

\[
\text{Protein Intake} = \text{Total feed consumed \times Crude protein in feed \times 100}
\]

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 ex-
perimental 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 %.

<table>
<thead>
<tr>
<th>Proximate components (%)</th>
<th>Control (0 %)</th>
<th>ZOP1 (1 %)</th>
<th>ZOP2 (2 %)</th>
<th>ZOP3 (3 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>10.50</td>
<td>10.98</td>
<td>9.86</td>
<td>9.56</td>
</tr>
<tr>
<td>Crude protein</td>
<td>40.01</td>
<td>40.00</td>
<td>40.04</td>
<td>39.98</td>
</tr>
<tr>
<td>Fibre content</td>
<td>3.10</td>
<td>3.12</td>
<td>3.04</td>
<td>3.42</td>
</tr>
<tr>
<td>Ash</td>
<td>4.50</td>
<td>4.45</td>
<td>3.95</td>
<td>3.74</td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.42</td>
<td>5.20</td>
<td>4.98</td>
<td>4.70</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>35.77</td>
<td>36.25</td>
<td>38.13</td>
<td>38.60</td>
</tr>
</tbody>
</table>

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 vary-
ing 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 sup-
plementation and the control is shown in table 6. There were differ-
ces 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 com-
pared 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 1 %
Z. officinale root-powder while the lowest FCR (1.57) was observed in
fish fed with control diet. There were significant no differences in the pro-
tein 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. offi-
cinale 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 in-
testine of fish fed varying level of Z. officinale root-powder are shown in tables 8 and 9.
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 Faturori [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 Obinbi et al. [26], suggested that ginger and garlic supplements collectively or individually improved growth performance of broilers. This was corroborated by Haghhighi 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.
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).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (0 %)</th>
<th>ZOP1 (1 %)</th>
<th>ZOP2 (2 %)</th>
<th>ZOP3 (3 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>2.30 ± 0.06*</td>
<td>2.33 ± 0.09*</td>
<td>2.33 ± 0.03*</td>
<td>2.33 ± 0.09*</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>14.22 ± 1.12*</td>
<td>20.27 ± 0.92*</td>
<td>18.49 ± 1.09*</td>
<td>20.14 ± 1.15*</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>11.92 ± 1.16*</td>
<td>17.95 ± 0.90*</td>
<td>16.15 ± 1.06*</td>
<td>17.82 ± 1.07*</td>
</tr>
<tr>
<td>Percentage weight gain (%)</td>
<td>518.3 ± 10.21*</td>
<td>840.8 ± 7.70*</td>
<td>691.4 ± 10.63*</td>
<td>762.3 ± 17.69*</td>
</tr>
<tr>
<td>Feed intake (g / fish)</td>
<td>19.17 ± 2.29*</td>
<td>27.11 ± 1.49*</td>
<td>25.2 ± 1.65b</td>
<td>25.64 ± 1.96a</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.40 ± 0.07*</td>
<td>1.57 ± 0.04*</td>
<td>1.56 ± 0.02b</td>
<td>1.55 ± 0.02b</td>
</tr>
<tr>
<td>Specific growth rate (%)</td>
<td>2.31 ± 0.19*</td>
<td>2.57 ± 0.10*</td>
<td>2.46 ± 0.06b</td>
<td>2.56 ± 0.02b</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.80 ± 0.09*</td>
<td>1.65 ± 0.04d</td>
<td>1.60 ± 0.02b</td>
<td>1.61 ± 0.07b</td>
</tr>
<tr>
<td>Apparent net protein utilization (%)</td>
<td>56.29 ± 1.38*</td>
<td>83.64 ± 3.92*</td>
<td>68.270 ± 5.12c</td>
<td>65.60 ± 5.05c</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>86.67 ± 8.82*</td>
<td>80.00 ± 5.77e</td>
<td>63.33 ± 3.33c</td>
<td>63.33 ± 3.33c</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control 0 %</th>
<th>ZOP1 1 %</th>
<th>ZOP2 2 %</th>
<th>ZOP3 3 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bacteria Count (CFU / ml) × 10^5</td>
<td>23.67 ± 0.88*</td>
<td>17.33 ± 4.76*</td>
<td>7.4 ± 5.31*</td>
<td>8.84 ± 7.58*</td>
</tr>
<tr>
<td>Total Fungal Count (CFU / ml) × 10^5</td>
<td>7.67 ± 0.44*</td>
<td>2.83 ± 1.36*</td>
<td>2.00 ± 0.00*</td>
<td>2.50 ± 0.00*</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Isolates Details</th>
<th>A1</th>
<th>B1</th>
<th>C1</th>
<th>D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Creamy</td>
<td>Dull Cream</td>
<td>Dull Cream</td>
<td>White</td>
</tr>
<tr>
<td>Shape / Margin</td>
<td>Irregular</td>
<td>Entire</td>
<td>Entire</td>
<td>Entire</td>
</tr>
<tr>
<td>Arrangement</td>
<td>Cocci in long chain</td>
<td>Baccilli</td>
<td>Baccilli</td>
<td>Cocci in pair</td>
</tr>
<tr>
<td>Surface Appearance</td>
<td>Smooth and Glistening</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth and Glistening</td>
</tr>
<tr>
<td>Elevation</td>
<td>Raised</td>
<td>Raised</td>
<td>Raised</td>
<td>Raised</td>
</tr>
<tr>
<td>Texture</td>
<td>Dry</td>
<td>Moist</td>
<td>Moist</td>
<td>Dry</td>
</tr>
<tr>
<td>Opacity</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Isolates Details</th>
<th>A2</th>
<th>B2</th>
<th>C2</th>
<th>D2</th>
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<tbody>
<tr>
<td>Colour</td>
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<td>Creamy</td>
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<td>White</td>
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<td>Shape / Margin</td>
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<td>Entire</td>
<td>Entire</td>
</tr>
<tr>
<td>Arrangement</td>
<td>Cocci in chain</td>
<td>Baccilli</td>
<td>Baccilli</td>
<td>Cocci in pair</td>
</tr>
<tr>
<td>Surface Appearance</td>
<td>Smooth and Glistening</td>
<td>Smooth and Glistening</td>
<td>Smooth and Glistening</td>
<td>Smooth and Glistening</td>
</tr>
<tr>
<td>Elevation</td>
<td>Raised</td>
<td>Raised</td>
<td>Raised</td>
<td>Raised</td>
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<td>Texture</td>
<td>Moist</td>
<td>Moist</td>
<td>Moist</td>
<td>Dry</td>
</tr>
<tr>
<td>Opacity</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
<td>Translucent</td>
</tr>
</tbody>
</table>

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 subtilis*, *Streptococcus agalactiae* and *Enterococcus faecalis*. This could indicate that *Z. officinale* root-powder favours or promotes the growth of gram positive bacteria in the intestine of *C. gariepinus*. 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 because mostly the bacteria flora in the intestine of *C. gariepinus* has been reported to stimulate only non-specific immune responses and suggested that the medicinal plants could be used to treat diseases. This gastrointestinal microbes also prevent colonization of the fish by other microbes that might otherwise be pathogenic. *B. subtilis* has been reported to promote performance and immune responses which might have taken place in fish fed ZOP1 and ZOP3. Tannins and alkaloids 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 as the level of inclusion (0.5 %, 1.0 % and 2.0 %) increased and as the months of the supplemented groups (onion bulb and walnut leaves) as the level of inclusion increased. The enterobacteriaceae load in the intestine of *C. gariepinus* consisted of *Bacillus subtilis*, *Streptococcus agalactiae* and *Enterococcus faecalis*. This is corroborated by Nwabueze [11] 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 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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Gram</th>
<th>Spores</th>
<th>Motility</th>
<th>Catalase</th>
<th>Starch hydrolysis</th>
<th>Gelatin liquefaction</th>
<th>Oxidase</th>
<th>Mannitol</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Glucose</th>
<th>Suspected organism</th>
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<tr>
<td>A1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Streptococcus agalactiae</td>
</tr>
<tr>
<td>A2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>C1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>C2</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>B. subtilis</td>
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<tr>
<td>D1</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>D2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>E. faecalis</td>
</tr>
</tbody>
</table>

**Table 10:** Biochemical test of the Bacteria isolates

**Table 11:** Fungal isolates from fish Intestine (1st replicate)

**Table 12:** Fungal isolates from fish intestine (2nd replicate).
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 anti-fungal. 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 anti-fungal 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.

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