



## Research article

# Effect of dietary cinnamon supplementation on the growth in length, haematology, serum biochemistry, intestinal microbiota and enzymes and histomorphological changes of the intestine, liver and the kidney of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂)

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## Abstract

In a 56-day feeding trial, the impact of dietary cinnamon supplementation on juvenile *Heteroclarias* (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) was examined. Five experimental diets with varying levels of cinnamon powder (ranging from 0% to 2.0%) were formulated. The study found that cinnamon supplementation positively influenced the fish's growth, haematology, serum biochemistry, intestinal microbiota, and enzyme activities. The group fed with 1.5% cinnamon (DT4) showed the highest growth performance, haematological indices, antioxidant, and hindgut enzyme activities. Additionally, the cinnamon-fed groups exhibited a significant reduction in total cholesterol levels, except for the 0.5% cinnamon-fed group (DT2). Blood glucose, blood urea nitrogen, and creatinine levels also decreased significantly in the cinnamon-fed groups compared to the control. Cinnamon supplementation increased bacterial counts, but fungi counts showed diverse effects, with the most favourable diversity indices observed in the 1.5% cinnamon group (DT4). Amylase, cellulase, and trypsin activity in the cinnamon-fed group were significantly higher than in the control group. Notably, all cinnamon-fed groups' intestine, liver, and kidney microstructures remained well-preserved, without any signs of injury. This study demonstrates that cinnamon has the potential to enhance the growth and overall well-being of African catfish hybrids. The optimal outcomes were observed when cinnamon was supplemented at a level of 1.5%, which can have significant implications for improving aquaculture practices and fish health.

**Keywords:** Cinnamon, *Heteroclarias*, trypsin, globulin, relative growth rate

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## INTRODUCTION

The fastest-growing food industry in the world is still aquaculture, which is an undisputed truth; a sustainable solution to the world's problems of hunger, malnutrition, and declining health among the large human population has been identified as aquaculture (FAO 2021). It has been observed that aquaculture is a business that produces significant profits globally and has one of the most important food sources (Brummett et al. 2008, Henriksson et al. 2021, See et al. 2021). Innovation in breeding programs has been one of the most obvious drivers of this growth (Afewerki et al. 2022, Lebel et al. 2021). The hybridization of catfish species led to a variety in the production in the catfish sector whereby hybrid catfish production is spreading around the globe and becoming endemic in places like Africa, Asia, Europe, and the USA (Porto-Foresti et al. 2013). There are assertions that hybrid catfish are preferable to native catfish in terms of their improved aquaculture performances, including reduced feed conversion ratios, improved immunological responses, and increased metabolic enzyme activities (Aiyelari and Adeyeye 2022, Zhang et al. 2020). Aquaculture environments are subject to a variety of stressors, such as changing climatic conditions that can cause hyperthermia (Khieokhajonkhet et al. 2022), aquaculture intensification that can cause crowding stress (Hanke et al. 2020, Amano et al. 2022), and changes in the microbiome of these environments that can affect the gut microbial community in fish (Chen et al. 2022). All of these pose cumulative risks to aquaculture health, endangering aquaculture operations. Fish farmers frequently add chemical additives or antibiotics to fish meals to treat or prevent disease and to increase feed utilization to keep up with the speed of aquaculture output (He et al. 2016). Antimicrobial efficacy has, however, significantly decreased as a result of growing worries about the emergence of antibiotic resistance strains (Dong et al. 2021). The presence of antibiotic residue in aquaculture products has raised questions about food safety in addition to the hazard that these strains pose through the transmission of resistant genes to human infections (Rigos and Kogiannou 2023). Approximately 1.27 million deaths in 2019 alone were due to antimicrobial resistance (AMR) (Murray et al. 2022). Due to the increasing demand for sustainable aquaculture products from the general public, it is becoming increasingly difficult to disregard the long-term effects of antibiotic use on aquaculture sustainability (Okeke et al. 2022). The growing initiatives to cut back on chemical antibiotic use in the fish industry sectors, therefore, prompts a question regarding the necessity of finding greener prophylactic and therapeutic choices (Easwaran et al. 2022). As a result of this, the identification of functional feed additives with organic origins has received increased attention recently. A substantial and growing body of literature has extensively discussed the potential utilization of medicinal herbs in fish feed. For a comprehensive review on this topic, refer to the works of Dawood et al., (2018), Zhu, (2020), and Reverter et al., (2021). These studies provide valuable insights and analysis regarding the use of medicinal herbs in fish feed, contributing to our understanding of their benefits and applications in the aquaculture industry (Dawood et al. 2018, Zhu 2020, Reverter et al. 2021). It is conceivable for medicinal plants to influence digestive enzymes, regulate immune responses, and boost antioxidant defenses against oxidative

stress, improving fish growth and nutrient retention due to the presence of the phytogenic substances in them (Alagawany et al. 2021). They are natural items that do not harm the environment, people's health, or fish health, which is the main advantage of utilizing them (Doan et al. 2020, Gabriel 2019). When compared to synthetic substances, the beneficial effects of phytochemicals are frequently equal to or even superior (Singh and Gaikwad 2020, Zhu 2020). Medicinal plants are environmentally safe, affordable, biodegradable, and recyclable (Caipang et al. 2021, Caipang et al. 2019).

Cinnamon (*Cinnamomum zeylanicum* L. and *Cinnamomum cassia* L.) is a species of the Lauraceae family and contains significant levels of numerous phytochemicals of biological importance. Flavonoids, saponins, phenols, manganese, iron, dietary fibre, calcium, and other beneficial substances and elements are also present in cinnamon (Kowalska et al. 2021, Heshmati et al. 2021). It has also been claimed to be a rich source of polyphenols and antioxidants, with anti-inflammatory, antidiabetic, antibacterial, and anticancer characteristics, making it a potentially beneficial contributor to both human and animal health (Błaszczuk et al. 2021). Other significant components of cinnamon include coumarin, cinnamyl alcohol, cinnamaldehyde, cinnamic acid, eugenol, and cinnamyl acetate (Wang et al. 2013). Cinnamon leaf or bark powder, or their oil or extract has been successfully included in the diets of; rainbow trout (*Oncorhynchus mykiss*) (Ravardshiri et al. 2021), European sea bass (*Dicentrarchus labrax*) (Habiba et al. 2021), pangasius catfish (*Pangasianodon hypophthalmus*) (Setiawati et al. 2016), Nile tilapia (*Oreochromis niloticus*) (Rahmawati and Ubaidillah 2017, Abdel-Tawwab et al. 2018), Monosex Nile tilapia (*Oreochromis niloticus*) (Amer et al. 2018), grass carp (*Ctenopharyngodon idella*) (Ghafoor 2020). All reported the efficacy of cinnamon in improving feed utilization, growth, health status and immunity. As far as our knowledge can reach the use of cinnamon in the diets of African catfish or its hybrid is still at its infancy if at all it exists. This study, therefore, investigated the effect of dietary cinnamon supplementation on the growth, haematology, serum biochemistry, intestinal microbiota and enzymes and histomorphological changes of the intestine, liver and the kidney of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂)

## MATERIALS AND METHODS

### Experimental Diet

The ground cinnamon bark powder and other ingredients for the feed were acquired from commercial vendors, processed, and individually screened to fine particle size. Following the procedure (AOAC 2010), the proximate composition of each feedstock was determined in triplicate. Based on the results of the ingredients' proximate analyses, a diet formulation tool designed by the Network of Aquaculture Centers in Asia-Pacific was used to develop the diets (NACA 2008). The concentrations of cinnamon powder used in the dietary ingredient mixture were 0% (DT1), 0.5% (DT2), 1.0% (DT3), 1.5% (DT4), and 2.0% (DT5) (Table 1). The mixture was moistened with hot water before being pressed through a mesh (2 mm Ø die) to form pellets, which were then dried in the sun at 30-32 °C for 72 hours. After being dried to a final

moisture level of less than 10%, the experimental diets were placed in a Ziploc bag and kept in a refrigerator (4 °C). Representative dietary treatments were collected for proximate analysis to confirm that the diets' desired protein and lipid levels were met.

**Table 1** Ingredient and nutrient composition (g/100g as fed basis) of the experimental diets

<i>Ingredient Composition (%)</i>	<b>DT1</b>	<b>DT2</b>	<b>DT3</b>	<b>DT4</b>	<b>DT5</b>
Fishmeal@72%cp	27.80	27.80	27.80	27.80	27.80
SBM@44% cp	44.50	44.50	44.50	44.50	44.50
Yellow Maize@10%cp	4.00	4.00	4.00	4.00	4.00
Cinnamon	0.00	0.50	1.00	1.50	2.00
Fish Premix	5.00	5.00	5.00	5.00	5.00
Fish oil	2.50	2.50	2.50	2.50	2.50
Soy Oil	2.50	2.50	2.50	2.50	2.50
Starch	13.70	13.20	12.70	12.20	11.70
<i>Proximate Composition (%)</i>					
Moisture	8.14	8.12	8.10	8.08	8.06
Crude Protein	37.82	38.00	38.19	38.38	38.58
Crude Lipid	12.31	12.40	12.50	12.59	12.69
Ash	7.39	7.42	7.44	7.47	7.50
Crude Fibre	5.91	5.92	5.93	5.94	5.95
NFE	28.44	28.14	27.84	27.54	27.24
<i>Amino Acid Composition (%)</i>					
Arginine %	3.00	3.01	3.03	3.04	3.06
Histidine	0.94	0.94	0.94	0.95	0.95
Isoleucine	1.84	1.85	1.85	1.86	1.87
Leucine	3.00	3.01	3.03	3.04	3.06
Lysine	2.82	2.83	2.85	2.86	2.88
Methionine	0.86	0.87	0.87	0.88	0.88
M+C	1.38	1.39	1.39	1.40	1.41
Phenylalanine	1.76	1.77	1.78	1.79	1.80
P+T	3.05	3.07	3.08	3.10	3.11
Threonine	1.78	1.79	1.80	1.81	1.82
Tryptophan	0.47	0.47	0.47	0.47	0.48
Valine	2.00	2.01	2.02	2.03	2.04

Each kg of the Agri-mix fish premix contains Vitamin A 1500000 i.u, Vitamin B1 800mg; Vitamin B2 1,600mg, Vitamin B6 1,500mg, Vitamin B12 4mg, Vitamin D3 3,000,000 i.u, Vitamin E 200000mg, Vitamin C 240g, Folic Acid 300mg, Niacin 5970 mg, Biotin 40 mg, Pantothenic Acid 4000mg, Copper 800mg, Iodine 191mg, Iron 1200mg, Manganese 20,323mg, Selenium 60mg, Zinc 15996 mg, Choline 87000mg. Manufactured by Agri-Dom 20/22 Kolawole Shonibare Street, Ajao Estate, Lagos. [www.agri-domintegrated.com.ng](http://www.agri-domintegrated.com.ng)

M+C: Methionine + Cysteine

P+T: Phenylalanine + Tyrosine

NFE: Nitrogen Free Extract

## Biochemical analysis

Following the procedure of AOAC (2010), the samples were heated to 105 °C for 24 hours in a Memmert oven to assess their moisture content. The amount of ash in the sample was determined by burning the samples in a furnace (Omegalux LMF-3550) at 600 °C for four hours. Using a Kjeldahl protein auto-analyzer (Foss Tecator Kjeltect™ 8400), the crude protein of the sample was assessed following acid digestion. The nitrogen was multiplied by 6.25 to produce crude protein. The crude lipid of the sample was measured using apparatus for Soxhlet extraction (Foss Tecator Soxtec™ 8000). The raw fibre was examined using a hot extraction fibre analyzer (Foss Tecator Fibertec). Trial data were analyzed in triplicate.

## Rearing Condition

The work was conducted in the wet laboratory of the Aquaculture and Fisheries Department at the University of Ilorin, Nigeria. African catfish hybrid (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂), Heteroclarias, fingerlings were sourced from a reputable hatchery in Ilorin, Nigeria and fed a standard diet (1.8 mm Skretting® catfish starter feed) while being exposed to laboratory setting for 15 days to acclimate. After acclimatization, 15 fish per replicate tank were measured to the nearest 0.1 cm (8.55 ± 0.12 cm average length) and assigned to the five dietary treatments in three replications. The fish were not fed for the entire day before the feeding experiment to prepare their digestive systems for the experimental diet, improve their hunger for the new diet, and lessen stress during length measurement and stocking. The fifteen 84-L rectangular plastic tanks (80 × 35 × 30 cm) were gently aerated after being filled with water to a capacity of 70 litres. For 56 days, each fish group was manually fed two times every day at 9:00 am and 5:00 pm. The bulk weighing was conducted every two weeks to adjust diet and monitor growth patterns. During the feeding trial, the water quality ranges of 27.26–28.12°C temperature, 6.55–6.75 mg/L dissolved oxygen, and 6.57–7.25 pH were maintained.

## Growth assessment

The underlisted variables were computed, using the techniques given by Lugert et al. (2016), for growth assessment.

$$\text{Absolute Growth (cm)} = \text{Final Length} - \text{Initial Length}$$

$$\text{Relative Growth Rate (\%)} = \frac{(\text{Final Length} - \text{Initial Length})}{\text{Initial Length}} \times 100$$

$$\text{Specific Growth Rate (\% d}^{-1}\text{)} = \frac{\ln(\text{Final Length}) - \ln(\text{Initial length})}{\text{culture period (day)}}$$

$$\text{Thermal Growth Coefficient} = \frac{\text{Final Length} - \text{Initial Length}}{\text{Temperature} \times \text{Period of Culture}}$$

$$\text{Dressing percentage (\%)} = \frac{(\text{Total weight} - \text{Viscera weight})}{\text{Total Weight}} \times 100$$

$$\begin{aligned} \text{Survival Rate (\%)} \\ = \frac{\text{Number of fish at the end of the feeding trial}}{\text{Number of fish at the beginning of the feeding trial}} \times 100 \end{aligned}$$



### Condition factor and body indices

The condition factor (K) and body indices were determined following the procedures explained in (Jimoh et al. 2022b)  $K = \frac{W}{L^3} \times 100$   
 W: Final weight of each fish to the nearest 0.01g.  
 L: Final Length of each fish to the nearest 0.1cm

Body indices were calculated as follows:

$$\text{Hepatosomatic Index (HSI, \%)} = \frac{\text{Liver weight}}{\text{whole body weight}} \times 100$$

$$\text{Viscerosomatic Index (VSI, \%)} = \frac{\text{Viscera weight}}{\text{whole body weight}} \times 100$$

### Blood sampling for haematology and serum biochemistry

Six fish from each test tank were taken out after the feeding period to have their blood analyzed. Earlier, the fish were gently euthanized using a solution of clove oil (100 mgL<sup>-1</sup>). A disposable 2 mL syringe and a 23G needle were used to extract 1 mL of blood from the caudal vein, which was then collected in an EDTA-treated container for a 3-part full auto haematology analyzer's haematological analysis (Model BK VET 200 mini). In plain sampling bottles, 2.5 mL of blood from fish given the various dietary treatment was taken. At 4 °C, the blood was allowed to coagulate. The coagulated blood samples were centrifuged for six minutes at 8,000 rpm to get serum after 30 minutes of coagulation for serum biochemistry analysis: total protein, albumin, total cholesterol, blood glucose, blood urea nitrogen, creatinine, serum alanine aminotransferases (ALT), serum aspartate aminotransferases (AST), serum alkaline phosphatase (ALP) etc.

### Serum electrolytes and oxidative stress biomarkers

An atomic absorption spectrophotometer was used to measure the serum electrolytes (K<sup>+</sup>, Cl<sup>-</sup>, Na<sup>+</sup>, and Ca<sup>2+</sup>). By following the published manufacturer's methods, commercial kits (Randox™, United Kingdom) were used to measure the serum stress indicators spectrophotometrically. These were carried out at the Tanke Ilorin Central Laboratory.

### Microbial Count

Aseptically dissected fish samples (n=6 fish distal intestines/treatment) from the various dietary groups had their hindguts removed, and they were then placed in a sterile vial with 5 mL of sterile distilled water. The contents of the bottle were violently shaken to aid in the contents' separation into the water. Before this, the working surface was thoroughly cleaned with pure alcohol, and the glassware was sterilized for 90 minutes at 160 °C. The techniques of Onions et al. (1981) were applied for fungal isolation. Total fungal counts were expressed in spore-forming units (SFU)/g. Throughout the identification of bacterial isolates, standard operating procedures were adhered to for the gram reaction, morphology, motility, catalase and oxidase reactions, citrate consumption, coagulase production, starch hydrolysis, and sugar (Claus 1992, Harrigan and McCance 1976, Seeley Jr and VanDemark 1962). Using the criteria laid out by Holt et al. (1994), the resulting colonies were verified. The

number of bacteria colonies that form after incubation was expressed using colony-forming units (CFUs)/g. After incubation at 37 °C for 24 hours, the number of bacterial colonies was expressed as colony-forming units (CFU)/g.

### Diversity Study

The following diversity indices were used to examine the microbial diversity in the hindgut of *Heteroclinarias* fed varied diets containing cinnamon.

$$\text{Simpson Index } (D) = \sum_{i=1}^s P^2$$

$$\text{Shannon Weiner Index } (H) = - \sum_{i=1}^s P \ln P$$

$$\text{Simpson Dominance Index } (1 - D) = 1 - D$$

$$\text{Margalef Richness Index} = \frac{(S - 1)}{\ln N} \quad \text{Simpson Diversity Index } (1/D) = \frac{1}{\sum_{i=1}^s P^2}$$

where S= total number of species

N= total number of individuals in the sample

### Intestinal Enzymes

The hindguts of six fish from each replicate were taken out, placed in sterile bottles with labels, and weighed. Using a motor-driven homogenizer with Teflon coating, each hindgut sample was homogenized in cool 0.25M sucrose. The homogenate was centrifuged at 5000 ×g for 15 minutes in a cooling centrifuge (5 °C); the supernatant was then taken out, put in sample vials, and frozen at -20 °C for the measurement of digestive enzymes. Amylase and lipase activity were tested by the method described by [Dar et al. \(2021\)](#), whereas protease activity was assessed following [Allameh et al. \(2017\)](#). The trypsin and chymotrypsin activity was measured using [Kunitz \(1947\)](#) casein digestion method.

### Intestine, liver and kidney histomorphology

Fish (n = 3/replicate) were anaesthetized with clove oil (100 mgL<sup>-1</sup>) after the experiment. A 10% potassium-buffered formalin solution was used to preserve their intestine, liver, and kidney. Using [Huges and Perry \(1976\)](#)'s technique, the organs were dehydrated at alcohol concentrations of 50, 70, 90, and 100%. The organs were then sealed with melted wax. The tissues were divided into 5-7 µm sections using a rotatory microtome, and then Harris haematoxylin-eosin (H&E) stain was applied to them. A light microscope was used to view the stained slides.

### Ethical Statement

Ethics of animal research as contained in University of Ilorin, Ilorin, Nigeria's research policy on the use and care of animals were strictly followed. The experimental protocol and procedures have been approved by the Departmental Ethical Review Committee (DERC), Department of

Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, Nigeria (PROTOCOL ID: DERC/AQF/0019; DERC APPROVAL NUMBER: DERC/AQF/2020/1219).

### Statistical analysis

The results of the experiment were subjected to a one-way analysis of variance (ANOVA) and were presented as mean $\pm$ SE after passing the Levene test for homogeneity of variance. When a significant difference ( $p<0.05$ ) was noticed, Tukey's B tests were employed to assess changes between individual treatment means. IBM SPSS Statistics 20 was used to conduct each analysis.

## RESULTS

### Growth performance, body indices and survival rate

The growth performance of Heteroclarias (*Clarias gariepinus* ♀  $\times$  *Heterobranchus bidorsalis* ♂) fed cinnamon supplemented diet is presented in Table 2. The fish fed DT3 and DT4 had a significantly ( $p<0.05$ ) higher absolute growth rate, relative growth rate, specific growth rate, thermal growth coefficients and dressing percentage when compared with the control. Other test dietary groups shared statistical similarities ( $p>0.05$ ) with the control in all these parameters.

**Table 2** Growth performance of Heteroclarias (*Clarias gariepinus* ♀  $\times$  *Heterobranchus bidorsalis* ♂) fed cinnamon-supplemented diet

Parameter	Control	DT2	DT3	DT4	DT5
Initial length (cm)	8.96 $\pm$ 0.05 <sup>a</sup>	8.48 $\pm$ 0.40 <sup>a</sup>	8.34 $\pm$ 0.27 <sup>a</sup>	8.53 $\pm$ 0.34 <sup>a</sup>	8.42 $\pm$ 0.20 <sup>a</sup>
Final length (cm)	11.66 $\pm$ 0.03 <sup>c</sup>	12.07 $\pm$ 0.03 <sup>b</sup>	12.56 $\pm$ 0.09 <sup>a</sup>	12.80 $\pm$ 0.19 <sup>a</sup>	11.74 $\pm$ 0.15 <sup>c</sup>
Absolute growth rate (cm)	2.70 $\pm$ 0.06 <sup>b</sup>	3.59 $\pm$ 0.38 <sup>ab</sup>	4.21 $\pm$ 0.31 <sup>a</sup>	4.27 $\pm$ 0.45 <sup>a</sup>	3.32 $\pm$ 0.12 <sup>ab</sup>
Relative growth rate (%)	30.14 $\pm$ 0.83 <sup>c</sup>	42.89 $\pm$ 1.77 <sup>b</sup>	50.14 $\pm$ 1.93 <sup>a</sup>	50.55 $\pm$ 1.18 <sup>a</sup>	39.16 $\pm$ 0.50 <sup>b</sup>
Specific Growth Rate (% d <sup>-1</sup> )	0.47 $\pm$ 0.01 <sup>b</sup>	0.65 $\pm$ 0.06 <sup>ab</sup>	0.74 $\pm$ 0.04 <sup>a</sup>	0.78 $\pm$ 0.08 <sup>a</sup>	0.59 $\pm$ 0.06 <sup>ab</sup>
Thermal Growth Coefficient (10 <sup>-3</sup> )	1.78 $\pm$ 0.07 <sup>b</sup>	2.37 $\pm$ 0.25 <sup>ab</sup>	2.79 $\pm$ 0.21 <sup>a</sup>	2.82 $\pm$ 0.30 <sup>a</sup>	2.19 $\pm$ 0.08 <sup>ab</sup>
Dressing Percentage	73.39 $\pm$ 0.17 <sup>ab</sup>	73.55 $\pm$ 0.22 <sup>ab</sup>	74.59 $\pm$ 0.51 <sup>a</sup>	74.79 $\pm$ 0.39 <sup>a</sup>	73.08 $\pm$ 0.09 <sup>b</sup>
Viscerosomatic Index (VSI)	6.03 $\pm$ 0.04 <sup>a</sup>	4.55 $\pm$ 0.03 <sup>c</sup>	4.41 $\pm$ 0.03 <sup>d</sup>	3.95 $\pm$ 0.01 <sup>c</sup>	4.91 $\pm$ 0.01 <sup>b</sup>
Hepatosomatic Index (HSI)	0.89 $\pm$ 0.05 <sup>c</sup>	1.12 $\pm$ 0.02 <sup>b</sup>	1.25 $\pm$ 0.03 <sup>b</sup>	1.65 $\pm$ 0.04 <sup>a</sup>	0.94 $\pm$ 0.01 <sup>c</sup>
Condition Factor	0.68 $\pm$ 0.02 <sup>a</sup>	0.70 $\pm$ 0.01 <sup>a</sup>	0.74 $\pm$ 0.02 <sup>a</sup>	0.75 $\pm$ 0.01 <sup>a</sup>	0.74 $\pm$ 0.03 <sup>a</sup>
Survival Rate (%)	78.60 $\pm$ 0.02 <sup>c</sup>	80.92 $\pm$ 0.02 <sup>d</sup>	85.71 $\pm$ 0.02 <sup>b</sup>	92.87 $\pm$ 0.02 <sup>a</sup>	83.33 $\pm$ 0.01 <sup>a</sup>

Row values (mean $\pm$ Standard error; n=3) with different superscripts are significantly ( $p<0.05$ ; Tukey's-b) different from each other

The HSI of the cinnamon dietary group was significantly ( $p<0.05$ ) higher than the control whereas in contrast was the VSI; the cinnamon dietary significantly lower values than the control. There were no significant differences ( $p>0.05$ ) in the condition factor of the different dietary groups. The survival rate had significantly ( $p<0.05$ ) higher values among the cinnamon dietary groups when compared to the control. Fish group fed DT4; 1.5% cinnamon-based diet had the highest value of these growth performance parameters



## Haematological changes

Table 3 presents the haematology of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon-supplemented diets. The cinnamon dietary group has a significantly ( $p<0.05$ ) elevated level of red blood cells, haemoglobin, haematocrit and platelets when compared to the control. No significant variations ( $p>0.05$ ) were recorded in the mean cell volume and mean cell haemoglobin between the control group and fish fed cinnamon-based diets up to 1.5% supplementation. Fish fed DT2 had significantly ( $p<0.05$ ) lower mean cell haemoglobin concentration when compared to other test dietary groups that shared statistical similarities ( $p>0.05$ ) when compared to the control

**Table 3** Haematology of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon supplemented diets

Parameter	DT1	DT2	DT3	DT4	DT5
Red Blood Cell ( $\times 10^6/\mu\text{L}$ )	0.88±0.02 <sup>c</sup>	1.24±0.01 <sup>b</sup>	1.37±0.02 <sup>a</sup>	1.39±0.04 <sup>a</sup>	1.33±0.02 <sup>a</sup>
Haemoglobin (g/dL)	2.75±0.04 <sup>c</sup>	3.81±0.05 <sup>c</sup>	4.36±0.02 <sup>b</sup>	4.61±0.02 <sup>a</sup>	3.61±0.04 <sup>d</sup>
Haematocrit (%)	10.27±0.03 <sup>c</sup>	15.05±0.02 <sup>c</sup>	16.50±0.02 <sup>b</sup>	16.84±0.01 <sup>a</sup>	13.21±0.04 <sup>d</sup>
Mean cell volume (fL)	116.21±2.77 <sup>a</sup>	121.59±0.25 <sup>a</sup>	120.76±2.00 <sup>a</sup>	121.59±3.09 <sup>a</sup>	99.19±0.87 <sup>b</sup>
Mean cell haemoglobin (pg)	31.11±0.67 <sup>a</sup>	30.74±0.32 <sup>a</sup>	31.88±0.58 <sup>a</sup>	33.28±0.95 <sup>a</sup>	27.12±0.03 <sup>b</sup>
Mean cell haemoglobin concentration (g/dL)	26.78±0.26 <sup>a</sup>	25.28±0.32 <sup>b</sup>	26.40±0.16 <sup>a</sup>	27.36±0.10 <sup>a</sup>	27.34±0.23 <sup>a</sup>
Platelet (mcL)	6.24±0.02 <sup>c</sup>	7.90±0.02 <sup>c</sup>	9.03±0.06 <sup>b</sup>	9.99±0.03 <sup>a</sup>	7.25±0.05 <sup>d</sup>

Row values (mean±Standard error; n=3) with different superscripts are significantly ( $p<0.05$ ; Tukey's-b) different from each other

## Physiological responses

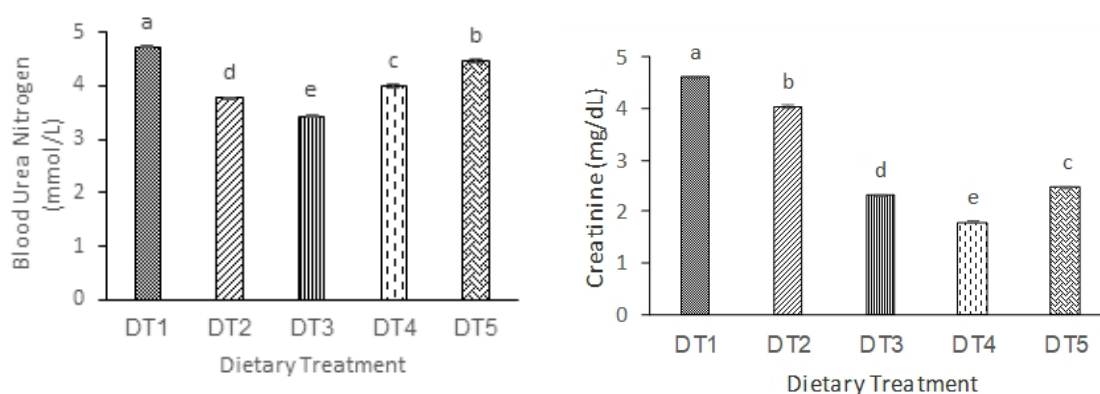
The DT3- and DT4-fed groups had significantly ( $p<0.05$ ) higher blood total protein and globulin contents than the control group (Table 4). When compared to the control, the test dietary groups' serum albumin contents and albumin-globulin ratios were considerably ( $p<0.05$ ) higher. The cinnamon-fed groups all, except the DT2-fed group, had significantly ( $p<0.05$ ) lower total cholesterol levels than the control group. When compared to the control group, the blood glucose level in the cinnamon-fed group decreases significantly ( $p<0.05$ ).

**Table 4** Physiological responses of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon-supplemented diet

Parameter	DT1	DT2	DT3	DT4	DT5
Total Protein (mg/dL)	4.71±0.03 <sup>b</sup>	4.18±0.03 <sup>c</sup>	8.52±0.02 <sup>a</sup>	8.50±0.03 <sup>a</sup>	3.39±0.07 <sup>d</sup>
Albumin (g/dL)	1.07±0.01 <sup>d</sup>	1.24±0.01 <sup>c</sup>	2.20±0.02 <sup>b</sup>	2.42±0.03 <sup>a</sup>	1.28±0.02 <sup>c</sup>
Globulin (g/dL)	3.64±0.02 <sup>c</sup>	2.95±0.04 <sup>d</sup>	6.33±0.03 <sup>a</sup>	6.09±0.03 <sup>b</sup>	2.11±0.06 <sup>c</sup>
Albumin/Globulin Ratio (g/dL)	0.30±0.01 <sup>d</sup>	0.42±0.01 <sup>b</sup>	0.35±0.01 <sup>c</sup>	0.40±0.01 <sup>b</sup>	0.61±0.02 <sup>a</sup>
Total Cholesterol	121.59±2.57 <sup>a</sup>	121.22±2.13 <sup>a</sup>	103.12±2.04 <sup>b</sup>	110.97±2.87 <sup>ab</sup>	108.90±4.96 <sup>ab</sup>
Glucose	103.50±3.81 <sup>a</sup>	86.56±5.62 <sup>b</sup>	81.74±3.81 <sup>b</sup>	78.18±1.80 <sup>b</sup>	88.19±1.79 <sup>b</sup>
<i>Serum Antioxidant Enzymes</i>					
Superoxide Dismutase (U/mL)	86.55±0.62 <sup>d</sup>	117.54±5.59 <sup>c</sup>	170.62±4.29 <sup>b</sup>	225.29±2.11 <sup>a</sup>	157.65±3.38 <sup>b</sup>
Catalase (U/mL)	421.25±3.90 <sup>c</sup>	645.07±4.69 <sup>c</sup>	683.59±5.24 <sup>b</sup>	1026.38±2.22 <sup>a</sup>	476.91±7.75 <sup>d</sup>
Glutathione Peroxidase (U/mL)	71.21±0.57 <sup>c</sup>	75.58±3.45 <sup>c</sup>	97.08±2.58 <sup>b</sup>	108.88±0.88 <sup>a</sup>	92.73±3.01 <sup>b</sup>
Glutathione S-Transferase (IU/L)	4.98±0.35 <sup>d</sup>	7.46±0.23 <sup>c</sup>	10.12±0.24 <sup>b</sup>	13.04±0.04 <sup>a</sup>	6.80±0.15 <sup>c</sup>

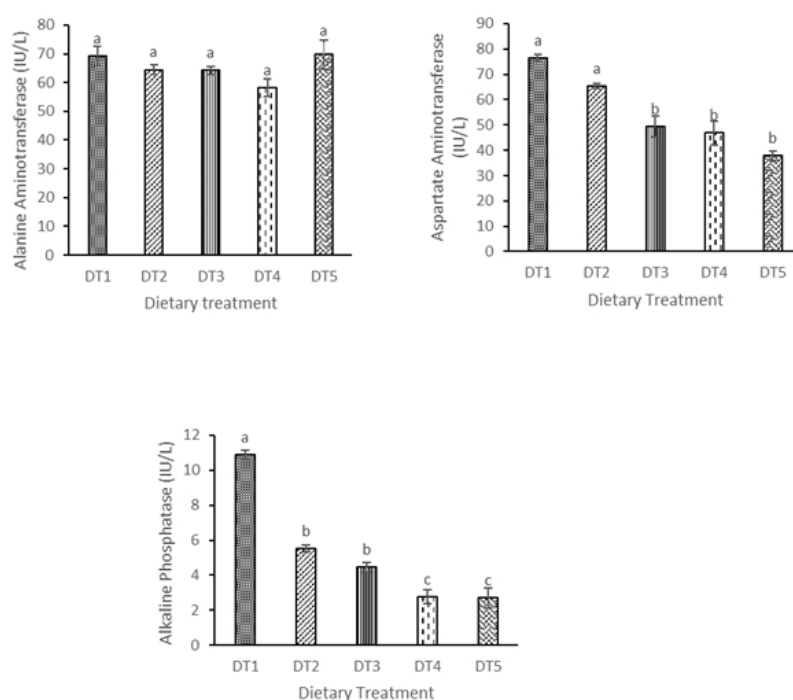
Row values (mean±Standard error; n=3) with different superscripts are significantly ( $p<0.05$ ; Tukey's-b) different from each other

With DT4-fed having the highest values of the various antioxidant enzyme activities parameters, the serum antioxidant enzymes activities were considerably ( $p < 0.05$ ) increased among the cinnamon-fed group when compared to the control. The blood urea nitrogen and creatinine recorded significantly ( $p < 0.05$ ) lower values among the cinnamon-fed groups than those in the control group (Figure 1)



**Figure 1** Kidney function test of Heteroclaris (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon-supplemented diet.

The serum alanine aminotransferases (ALT) were statistically similar ( $p > 0.05$ ) among the dietary groups. Except for DT2-fed groups that shared statistical similarities with the control group, other cinnamon-fed groups had significantly ( $p < 0.05$ ) lower serum aspartate aminotransferases (AST). The serum alkaline phosphatase (ALP) of the cinnamon dietary groups was significantly reduced ( $p < 0.05$ ) when compared to the control (Figure 2).



**Figure 2** Liver function test of Heteroclaris (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon supplemented diet.

### Serum electrolytes

The serum potassium ion of the cinnamon dietary groups was comparably ( $p < 0.05$ ) higher than the control (Table 5). Except for the DT4-fed group, the serum sodium ion of the different dietary groups was statistically similar ( $p > 0.05$ ). Serum chloride ions concentration of the cinnamon-fed groups significantly ( $p < 0.05$ ) reduced when compared to the control except in the DT4-fed group that shared statistical similarities with the two (control and test dietary groups). Serum calcium ion concentration was significantly reduced in a dose-dependent trend from the control.

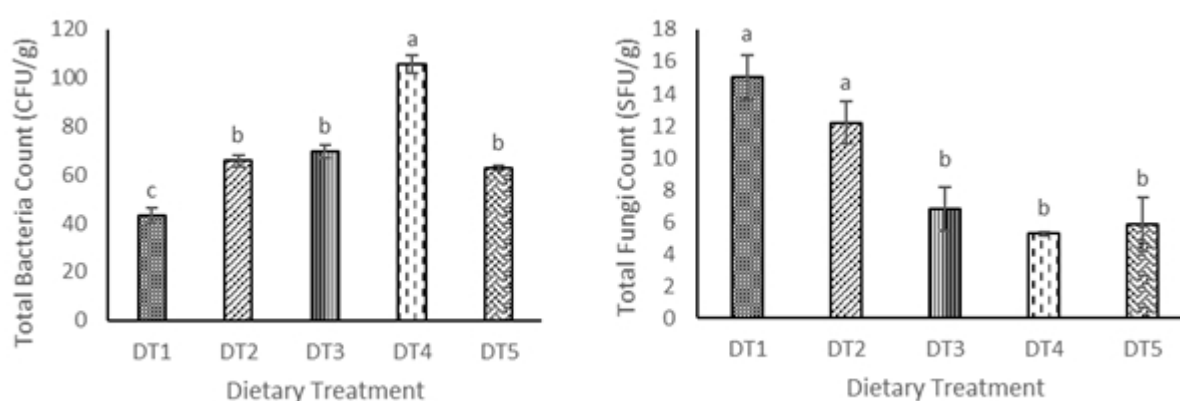
**Table 5** Serum electrolytes of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon-supplemented diet

Parameter	DT1	DT2	DT3	DT4	DT5
Potassium ion (mmol/L)	0.35±0.02 <sup>d</sup>	0.86±0.01 <sup>c</sup>	0.95±0.01 <sup>b</sup>	1.12±0.04 <sup>a</sup>	1.13±0.01 <sup>a</sup>
Chloride ion (mmol/L)	24.49±0.50 <sup>a</sup>	20.36±0.54 <sup>b</sup>	20.98±0.23 <sup>b</sup>	21.94±1.13 <sup>ab</sup>	16.80±0.52 <sup>c</sup>
Sodium ion (mmol/L)	7.19±0.82 <sup>b</sup>	8.37±0.18 <sup>b</sup>	9.59±0.79 <sup>b</sup>	13.47±0.15 <sup>a</sup>	9.41±0.63 <sup>b</sup>
Calcium ion (mg/dL)	5.05±0.25 <sup>a</sup>	4.87±0.10 <sup>a</sup>	3.48±0.11 <sup>b</sup>	1.68±0.10 <sup>c</sup>	1.52±0.19 <sup>c</sup>

Row values (mean±Standard error; n=3) with different superscripts are significantly ( $p < 0.05$ ; Tukey's-b) different from each other

### Intestinal microbial count and diversity

Figure 3 presents the hindgut microbial count of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon-based dietary treatments. Bacteria counts were heightened significantly ( $p < 0.05$ ) with cinnamon supplementation when compared to the control. the contrast was the results for fungi count; except for the DT2-fed group, the fungi count significantly reduced ( $p < 0.05$ ).



**Figure 3** Hindgut microbial count of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon-based dietary treatments .

The isolated bacteria were *Escherichia coli*, *Lactobacillus* spp., *Shigella* spp., *Salmonella* spp. and *Staphylococcus* spp. (Table 6). The diversity indices employed revealed that the DT3- and DT4-fed groups had more diverse bacteria species than other dietary groups,

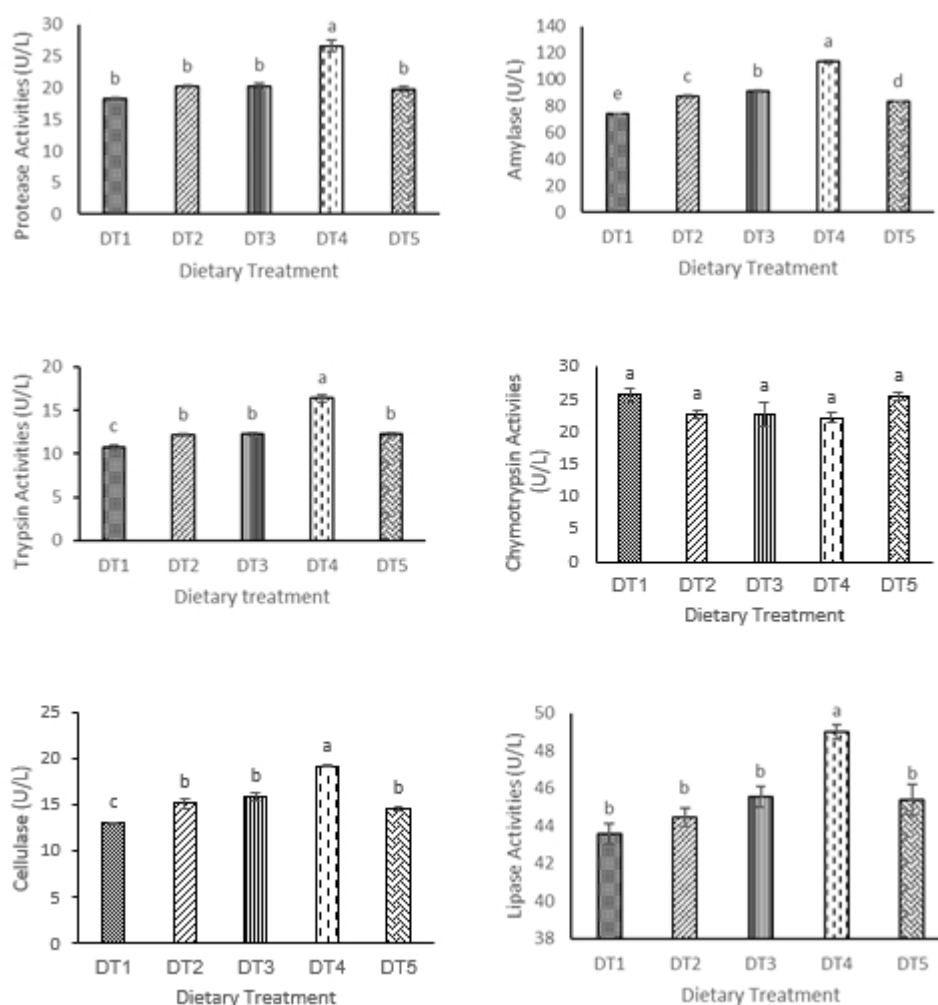
**Table 6** Microbial occurrence and diversity in the hindgut section of Heteroclaris (*Clarias gariepinus* ♀ × *Heterobranchius bidorsalis* ♂) fed cinnamon-based dietary treatments

Bacteria	Control	DT2	DT3	DT4	DT5
<i>Escherichia coli</i>	1 (0.50)	0 (0.00)	1 (0.33)	1 (0.20)	0 (0.00)
<i>Lactobacillus spp</i>	1 (0.50)	1 (0.50)	1 (0.33)	1 (0.20)	1 (0.50)
<i>Shigella spp</i>	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.20)	0 (0.00)
<i>Salmonella spp</i>	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.25)	0 (0.00)
<i>Staphylococcus spp</i>	0 (0.00)	1 (0.50)	1 (0.33)	0 (0.00)	1 (0.50)
<u>Diversity Indices</u>					
<i>Simpson Index (D)</i>	0.50	0.50	0.33	0.25	0.50
<i>Simpson Dominance (1-D)</i>	0.50	0.50	0.67	0.75	0.50
<i>Simpson Diversity Index (1/D)</i>	2.00	2.00	3.00	4.00	2.00
<i>Shannon-Weiner Index(H)</i>	1.69	1.69	1.10	1.39	1.69
<i>Margalef's richness</i>	5.77	5.77	3.64	2.89	5.77

Values in brackets are proportion

### Intestinal Enzymes

Figure 4 shows the hindgut intestinal enzyme activities of the fish fed the different dietary treatments. Except for the DT4-fed group that had significantly higher ( $p < 0.05$ ) values, protease and lipase activity were statistically comparable ( $p > 0.05$ ) across the various dietary groups. The amylase, cellulase and trypsin activity of the cinnamon-fed group was significantly ( $p < 0.05$ ) elevated when compared to those of the control group. No significant differences ( $p > 0.05$ ) were seen between the cinnamon-fed groups even though their chymotrypsin activity was lower than that of the control group.

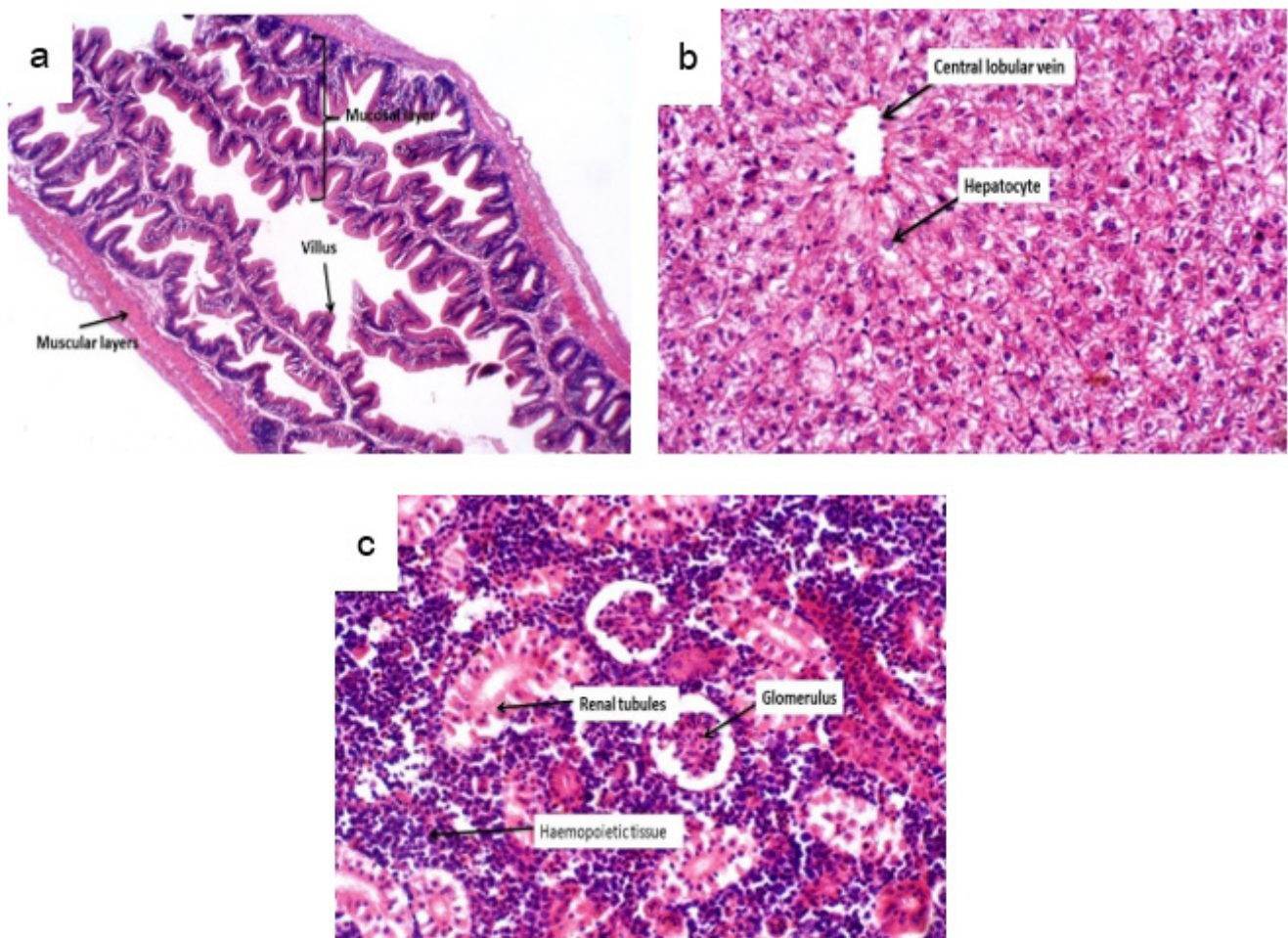


**Figure 4** Hindgut enzyme activities of Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon supplemented diet.

### Intestine, liver and kidney histomorphology features

The Heteroclarias (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed cinnamon based diets have their intestine, liver, and kidney histologically examined in Figures 5a–c. Figure 5a is an example of typical intestinal sections of the fish fed the different dietary treatments, showing the columnar epithelium and villous structures that make up the mucosal tissue. The layers of the muscle were also kept intact with no visible symptoms of lesions among the different dietary groups. Figure 5b is a typical liver section of the fish from the different dietary groups showing tissue with intact architecture composed of cords of healthy hepatocytes with lots of transparent vacuolated cytoplasm and regular round nuclei without indications of lesions recorded among them. Figure 5c displays a typical kidney section of the fish from the different dietary groups demonstrating tissue with a stable architecture made up of healthy glomeruli, renal tubules, and interstitial tissue predominantly composed of hemopoietic tissue. There were no indications of either an acute or a chronic injury among the different dietary groups.





**Figure 5** 5a-c. Representative histological details (5  $\mu$ m sectioned, H&E Stained, x100) of the intestine, liver and kidney of *Heteroclaris* (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂) fed cinnamon supplemented diets.

a. a typical section of the intestine, displaying mucosal tissue made up of villous structures and columnar epithelium. Additionally preserved were the muscle layers. No signs of injury could be seen.

b. a typical liver section displaying tissue with an intact architecture made up of cords of healthy hepatocytes with plenty of clear vacuolated cytoplasm and regular round nuclei without signs of injury.

c. a typical kidney section displaying tissue with an intact architecture made up of healthy glomeruli, renal tubules, and interstitial tissue primarily made up of hemopoietic tissue. There were no signs of an acute injury or a chronic injury.

## DISCUSSION

Cinnamon boosts immunity, health, and growth performance (Hussain et al. 2019, Begum et al. 2018). The results of this investigation validated the numerous potentials of cinnamon as a growth promoter, antioxidant, and gastrointestinal health booster. The fish fed DT3 (1% cinnamon) and DT4 (1.5% cinnamon) had a significantly ( $p < 0.05$ ) higher absolute growth rate, relative growth rate, specific growth rate, thermal growth coefficients and dressing percentage when compared with the control. Our report is in tandem with the findings of Ahmad et al. (2011) who administered cinnamon in the diet of Nile tilapia. The findings of this study were also supported by reports

for various other fish species such as in rainbow trout (*Oncorhynchus mykiss*) (Ravardshiri et al. 2021), European sea bass (*Dicentrarchus labrax*) (Habiba et al. 2021), pangasius catfish (*Pangasianodon hypophthalmus*) (Setiawati et al. 2016), Nile tilapia (*Oreochromis niloticus*) (Rahmawati and Ubaidillah 2017, Abdel-Tawwab et al. 2018), Monosex Nile tilapia (*Oreochromis niloticus*) (Amer et al. 2018), grass carp (*Ctenopharyngodon idella*) (Ghafoor 2020), Asian stinging catfish (*Heteropneustes fossilis*) (Begum et al. 2018). Fish group fed DT4; 1.5% cinnamon-based diet, had the highest value of these growth performance parameters. Habiba et al. (2021) observed that the European sea bass (*Dicentrarchus labrax*) fed a diet based on cinnamon had a significantly higher value of growth performance and survival rate similar to that found in the 1.5% dietary group in this investigation. Similar to what Mohammad (2021) observed about common carp (*Cyprinus carpio*) fed cinnamon-based diets. Begum et al. (2018), Abdel-Tawwab et al. (2018) and Ahmad et al. (2011) reported optimal growth performance measures with a diet that contained 1% cinnamon. Setiawati et al. (2016) also reported optimum growth performance at 1% cinnamon leaf powder supplementation in the diet of pangasius catfish (*Pangasianodon hypophthalmus*). Majid and Mousavi-Sabet (2022) reported that adding 1.2% cinnamon powder to the diet supported optimum health, feed utilization, and growth performance of rainbow trout fingerlings. The physiologically active phytochemicals found in cinnamon, which have been shown to support fish growth (Yang et al. 2015, Caipang et al. 2021, Caipang et al. 2019), may be a factor in the growth-promoting effect seen in the Heteroclaris fed cinnamon-based diets. Other test dietary groups in this study had higher values of these parameters but shared statistical similarities ( $p > 0.05$ ) with the control in some of the growth parameters. A plausible explanation for the enhancement of growth parameters among the cinnamon-fed groups when compared to the control might not be unconnected with the improved antioxidant capacity, enhanced digestive enzymes activity and boosted gut microbial populations brought about by the bioactive compound in cinnamon (Majid and Mousavi-Sabet 2022, Ravardshiri et al. 2021). The findings of our investigation in some ways supported this claim. Cinnamon contains a variety of phenolic components, including minerals, vitamins, essential oils including cinnamic and cinnamyl aldehydes, tannins, and saponins (Singh & Gaikwad, 2020). The metabolism may be boosted by tannins, flavonoids, and saponins because they may promote nutrient absorption (Dawood 2021, Rahmawati and Ubaidillah 2017). Flavonoids, which are antibacterial components of cinnamon, may increase the synthesis of endogenous digestive enzymes by reducing the population of harmful bacteria and boosting the population of good bacteria (Abd El-Hack et al. 2021). These phytochemical additives are dispersed throughout the body of the fish after ingestion, digestion, and absorption, where they have an impact on the physiological state of the fish.

Higher but statistically similar condition factor values recorded among the cinnamon dietary groups when compared to the control are an indication of comparable good environmental quality of the different groups under study. Similar observation was also made by Abbas et al. (2019). The morphological biomarkers (HSI, VSI, and Condition Factor) essentially confirm the fish's general health. The condition factor is used to assess the general health of fish as well as the quality of the water surrounding them, whereas the hepatosomatic

index (HSI) is used to provide information on the health of the fish (El-Agri et al. 2022, Xu and Jing 2012). The HSI of the cinnamon dietary group that was significantly ( $p < 0.05$ ) higher than the control group evidently points to an improved health condition of the fish in the cinnamon dietary groups. Contrast was the results of the VSI of the fish; the cinnamon dietary groups had significantly lower values than the control. This might be owing to phytochemical substances in cinnamon which deplete the fat tissue surrounding the visceral organ. Some phytochemicals guard against the body accumulating too much fat in its tissues by increasing the body's calorie burning rate. Records from this study where the cinnamon-fed groups all, except the DT2-fed group, had significantly ( $p < 0.05$ ) lower total cholesterol levels than the control group further revealed the hypocholesterolemic and hypolipidemic effect of cinnamon supplementation in the diet of fish. Yang et al. (2021) also reported these effects on grass carp (*Ctenopharyngodon idellus*) fed low fish meal diet. The survival rate that was significantly ( $p < 0.05$ ) higher in values among the cinnamon dietary groups when compared to the control further supported this assertion.

Haematological parameters have been viewed as a trustworthy indicator of the fish health status when assessing the overall health of fish to the prescribed dietary treatments (Jimoh et al. 2022a). The results of the three basic haematological tests (RBC, Hb, and haematocrit) used in this investigation showed patterns that complemented those of the growth performance parameters; the cinnamon dietary groups had a significantly elevated level of red blood cell, haemoglobin, haematocrit and platelets when compared to the control with DT4-fed group having the highest values of these parameters. An elevated level of these primary haematological measures has been linked to weight gain because increasing haemoglobin content has an immediate impact on fish respiratory gas transit, heart rate, and ultimate weight gain (Jawad et al. 2004, Majid and Mousavi-Sabet 2022, Montazeri Parchikolaei et al. 2021). Ahmad et al. (2011) reported that the number of RBC, haemoglobin, and hematocrit in Nile tilapia was significantly increased by adding 1% cinnamon powder to the diet. Ravardshiri et al. (2021) reported a non-significant increase in these parameters for rainbow trout fed cinnamon-supplemented carbohydrate diets. Jimoh et al. (2022c) made a similar observation when selected leafmeals were fed to hybrid carp (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂). In addition to their usual function in blood clotting, platelets are known to have a significant role in innate immunological and inflammatory responses (Holinstat 2017). The fish on cinnamon diets had greater platelet counts than the fish fed the control diet, demonstrating that they had stronger immunological responses. The immunostimulatory property of cinnamon is well documented (Aluwi et al. 2022), and our findings corroborated this claim.

Total protein, albumin, and globulin concentrations in serum can be considered as immunophysiological markers in fish (Soltani et al. 2017). These markers provide valuable information about the overall health and immune status of the fish (Fawole et al. 2023). Changes in the concentrations of these serum proteins can indicate alterations in the immune response and can be associated with various physiological and pathological conditions (Gao et al. 2021). They serve as indicators of immune system activation, inflammation, nutritional status, and certain diseases (Zhu et al. 2020). The DT3- and DT4-fed groups had significantly higher blood total protein and globulin contents than



the control group. Although albumin has a disproportionately higher impact on serum total protein levels, changes in albumin concentration are the main source of variations in serum total protein levels. Globulin might not change. Most scientists use the albumin-globulin ratio as a gauge of fish health and immunity (Mohammadiazarm et al. 2021). In this study, the serum albumin contents and albumin-globulin ratios of the cinnamon dietary groups were considerably ( $p < 0.05$ ) higher when compared to the control. Since the humoral components of the non-specific immune system are present in quantifiable proportions in serum concentrations of total protein, albumin, and globulin (Haghighi et al. 2017), it suffices to assert that the non-specific immune systems of the cinnamon dietary groups were strengthened by the significant amounts of these serum proteins among the dietary groups. Significantly elevated values of total protein and albumin were reported by Ravardshiri et al. (2021) and Majid and Mousavi-Sabet (2022). Albumin, a protein made in the liver that keeps blood from leaking from blood vessels, is a marker of liver functionality (Rashidi et al. 2020). Albumin facilitates the transport of hormones, drugs, vitamins, and other essential substances throughout the body. The statistically greater levels of albumin found among the cinnamon dietary groups in this study may suggest that cinnamon supplementation increased the liver's functionality and gave these dietary groups a higher capacity to stop blood from leaking from blood vessels. The liver function enzymes (ALT, AST and ALP) that were statistically similar or significantly lower to those obtained for control in this study further established the hepatoprotective effect of cinnamon supplementation empirically depicted by the liver sections of the fish from the different dietary groups having tissue with intact architecture without indications of lesions recorded among them. Yang et al. (2021) reported the hepatoprotective effect of apple polyphenol on grass carp (*Ctenopharyngodon idellus*). Those phytochemical compounds in cinnamon are clearly responsible for the effect recorded. Medicinal plants are known for this effect (Tadese et al. 2021, Yang et al. 2015).

The most popular herbal treatment for decreasing blood glucose and cholesterol is cinnamon (Kim et al. 2006, Sharma et al. 2020). Our findings established this hypoglycemic effect of cinnamon supplementation; the blood glucose level in the cinnamon-fed group decreases significantly when compared to the control group. Other authors supporting these conclusions included Kaur et al. (2019) and Ghafoor (2020). Glucose metabolism is activated and stimulated by polyphenols, a source of insulin mimics (Ghafoor 2020). Cortisol and glucose are recognized and often used markers of fish stress (Odhiambo et al. 2020). When a fish is exposed to a stressful scenario, internal tissue releases cortisol into the blood (Raposo de Magalhães et al. 2020). It stimulates the liver to create glucose by either glycogenolysis (the breakdown of glycogen to glucose) or gluconeogenesis (the breakdown of proteins to glucose) after it reaches the liver to meet the growing needs of the cells for energy (Li et al. 2022). Because glucose is an innate immunological parameter that is mediated by stress (Zheng et al., 2019), a rise in glucose concentration is a secondary reaction to stress, and the magnitude of the increase is a measure of stress. Hence the results of this study further established there was little or no stress situation recorded among the cinnamon dietary group. This may logically explain why lower serum total cholesterol levels were observed in the cinnamon-fed groups in this study. In addition to providing energy for cells, triglycerides and cholesterol

also serve as a marker for the presence of energy reserves. Fish use them as a source of energy while they are under stress situation (Prakash and Verma 2020). The mevalonate pathway, which manufactures cholesterol, depends on the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase (Baskaran et al. 2015). Baker et al. (2008) explained the mechanism behind this serum total cholesterol reduction by stating that cinnamon reduces hepatic HMG-CoA reductase activity, which reduces cholesterol formation in the liver, and suppresses lipid peroxidation. It is not unlikely that phytocomponents in cinnamon induced this HMG-CoA reductase inhibition. Due to the reduced cholesterol levels seen in the cinnamon dietary groups, the HMG-CoA reductase inhibitory activity was thus reported in this investigation in an indirect manner. Supplemental cinnamon has a direct impact on lipid metabolism, and as total cholesterol is a biomarker for lipid function, this enhances lipid-related blood biochemistry.

The significantly increased antioxidant capacity of the cinnamon dietary groups in this study as compared to the control further clearly demonstrates this low-stress condition among the dietary groups. Cinnamon's bioactive component may enable animals fed with it to have higher antioxidant capacities (Hamed et al. 2022). Jasim et al. (2022) reported higher antioxidant capacity in rainbow trout (*Oncorhynchus mykiss*) fed cinnamon-based diets. To inhibit lipid oxidation in pork, pig diets can be supplemented with 500 mg/kg of green tea extract (Norkeaw et al. 2022). The blood urea nitrogen and creatinine level are regarded as kidney function test biomarkers, their elevation in the blood suggests kidney damage (McDonald and Grosell 2006). The blood urea nitrogen and creatinine of the cinnamon-fed group decreases significantly when compared with the control. This conferred a reno/nephroprotective effect on the fish as evidently shown in the typical kidney section of the fish from the different dietary groups demonstrating tissue with a stable architecture where there were no recorded indications of either an acute or a chronic injury. There is evidence that some herbs, possibly because they contain bioactive compounds, can have reno/nephroprotective effects on fish (Huayue et al. 2022). Fish stress levels could be monitored using serum electrolytes as markers (Shui et al. 2018). The serum electrolytes that were statistically comparable to the control further showed no stress condition in fish of the different dietary groups. When serum calcium ion is significantly elevated, it may reduce the permeability of the blood cell membrane (Edori et al. 2013). The serum potassium ion of the cinnamon dietary groups was comparably higher than the control. The serum sodium and potassium ions are actively transferred between blood cells and plasma to maintain a healthy blood volume in the body and enhance physiological function (Limbaugh et al. 2021).

The gut health was significantly improved with cinnamon supplementation in this study. The bacteria counts were heightened significantly when compared to the control. *Escherichiacoli*, *Lactobacillus* spp., *Shigella* spp., *Salmonella* spp. and *Staphylococcus* spp. were the isolated organisms. Anaerobic bacteria, *Escherichia coli*, isolated from fish gut regions, have been shown to make a significant contribution to fish nutrition (Clements et al. 2009); they are known to generate amylase (Ray et al. 2007), lipase, and glycosidase (Ramirez and Dixon 2003). The isolated gram-negative bacteria in this study, *Shigella* spp., and *Escherichia coli*, have enzymes that can digest complex polysaccharides



(Ray et al. 2012). Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are known to be produced by them (Clements and Choat 1995, Clements 1997, Seeto et al. 1996, Stevens and Hume 2004). These SCFAs give room for the growth and proliferation of beneficial gut lactic acid bacteria such as lactobacillus. The growth of lactic acid bacteria could increase the health of a host (Gibson and Roberfroid 1995) by encouraging positive effects on cholesterol and glucose metabolism (Clements and Choat 1995, Gray 2006, Scott et al. 2008). Diversity indices were in favour of DT4 dietary group. This clearly explains why improved growth conditions were recorded among the dietary group (1.5% cinnamon). The presence of gut-associated bacteria in DT4 dietary group improves feed digestion and utilization by producing exogenous enzymes that can break down starch or cellulose. These microorganisms are known to serve a complementary function in the breakdown of fish feed (Banerjee et al. 2016, Bairagi et al. 2002). This explains why the DT4-fed group had significantly higher values of protease and lipase activity. The amylase, cellulase and trypsin activity of the cinnamon-fed group was significantly elevated when compared to those of the control group. Protease, amylase, and lipase activity in tilapia were increased by dietary cinnamon nanoparticles (Abdel-Tawwab et al. 2018). Cinnamaldehyde was demonstrated by Zhou et al. (2020) to increase the capacity for digestion and absorption by increasing the activities of intestinal, hepatopancreatic, and intestinal brush border digestive enzymes in grass carp (*Ctenopharyngodon idella*). Although the chymotrypsin activity of the cinnamon-fed groups was lower than that of the control group, no significant variations between the groups were observed, indicating that there was no growth reduction among the dietary groups. In rainbow trout, cinnamon induced higher production of these three digestive enzymes. (Ravardshiri et al. 2021). In contrast to trypsin, which is the primary protease enzyme during situations that promote growth, heightened chymotrypsin plays a prominent role in conditions that restrict or depress growth (Aderolu and Sahu 2015). The gastro-protective effect of cinnamon is evidenced in the hind intestine microstructures that were well preserved with no features of injury recorded in this study

## CONCLUSIONS

The aforementioned makes it clear that cinnamon has an empirically demonstrated ability to promote growth and health, with the best results being observed at a supplementation level of 1.5% cinnamon.

## CONFLICT OF INTEREST

The authors claim that no known conflicting financial or personal interests were clearly seen to interfere with the preparation of the study.

## AUTHOR CONTRIBUTIONS

**Wasiu Adeyemi Jimoh:** Conceptualization; Supervision; Writing – Final review & editing

**Ayodeji Ahmed Ayeloja:** Data curation; Formal analysis Investigation; Writing – review & editing

**Olayinka Abosede Ojo:** Data curation; Formal analysis; Writing – review & editing

**Comfort Timileyin Ayodele:** Project administration; Writing – original draft

**Adijat Ebunlomo Alabi:** Project administration; Writing – original draft

**Grace Amara Obinnakwelu:** Project administration; Writing – original draft.

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