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RESEARCH

Effects of Supplementation of Insoluble Dietary Fiber Obtained from Cinnamon Spent Bark Waste on the Performance of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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ABSTRACT

Dietary fiber supplementation has proven benefits on fish health and growth. Cinnamon spent bark waste is the cinnamon bark residue after oil distillation. It is a rich source of insoluble dietary fiber with very low contents of soluble dietary fiber. This study aimed to investigate the potential of using water-extracted insoluble fiber from cinnamon spent bark waste as a functional ingredient in the diets of *Oreochromis niloticus* fingerlings. Four experimental diets were prepared by replacing a commercial feed with extracted dietary fiber at 0 (control), 0.5%, 1%, and 1.5% levels. Fingerlings of *O. niloticus* were assigned to the four experimental diets and the feeding trial was conducted for 12 weeks. The results revealed that the weight gain, specific growth rate, feed conversion ratio, Fulton's condition factor, and survival rate of fish were not significantly different among the experimental groups. Fiber supplementation at 1.5% significantly increased ($p<0.05$) the total aerobic bacteria population in feces, whereas the coliform counts in feces at 0.5% and 1% fiber supplementation were significantly lower ($p<0.05$) than the control. There was a significant increase ($p<0.05$) in red blood cells count at 0.5% fiber supplementation. Moreover, insoluble dietary fiber supplementation significantly ($p<0.05$) increased the white blood cell counts in blood. Results suggested that insoluble fiber supplementation affected gut microbial populations and blood parameters of *O. niloticus* fingerlings. However, further investigations on gut microbiology and hematology are needed to ensure the use of insoluble dietary fiber from cinnamon spent bark waste as a functional ingredient in the diets of *O. niloticus* fingerlings.

INTRODUCTION

Dietary fiber is well known for its anti-inflammatory, antibacterial, and anti-cancerous effects in monogastric animals and hence, widely used as an additive in human and animal nutrition (Lin et al., 2020). Monogastric animals including fish are unable to digest fibrous carbohydrates such as cellulose (Li et al., 2012; Bonvini et al., 2018), because they lack fiber-digesting enzymes such as α-cellulase or microorganisms which digest fiber (Li et al., 2012; Wang et al., 2021). The inclusion of dietary fiber in low levels (3-5%) has positively impacted fish growth, however high levels of dietary fiber (>8%) have reduced the growth in certain fish species due to the reduced dry matter digestibility of the diet (Bonvini et al., 2018; Zhong et al., 2020). The addition of dietary fiber at optimum levels can enhance growth performance by improving immune response and prebiotic activity (Zhong et al., 2020). Prebiotic fiber encourages the growth and activity of beneficial bacteria (Tiengtam et al., 2015; Farzad et al., 2021) while inhibiting harmful bacteria in the intestinal tract. As a result, the health of the host is positively impacted (Tiengtam et al., 2015). However, the underlying mechanisms of health improvement effects of dietary fiber are not yet fully investigated (Zhong et al., 2020).

Nile Tilapia (*Oreochromis niloticus)* is considered as a favorable species to farm in tropical regions due to its high adaptability and fast growth rate. However, occasional disease outbreaks happen in summer seasons as water temperature increases lead to high losses (Tiengtam et al., 2015). In addition, huge losses have been reported in tropical countries after the stocking of low-quality Nile tilapia fingerlings obtained from hatcheries with poor conditions (Migiro et al., 2014). Antibiotics are the common chemotherapeutic agents used for disease control in aquaculture farms. However, excessive use of antibiotics can be problematic to public health and the balance in the ecosystem. The bio-therapeutic agents like prebiotics are a cost-effective and environmentally friendly substitute for antibiotics (Tiengtam et al., 2015). Application of phytogenic bioactive components into aquaculture feed has gained much attention due to the functionality of those in animal bodies (De la Cruz et al., 2023).

Ceylon cinnamon (*Cinnamomum zeylanicum*) has a high demand in the world cinnamon market. During cinnamon oil extraction and quills processing, the spent bark is disposed of as waste. Many of these food industrial by-products are rich sources of functional and novel dietary fibers (Sharma et al., 2016). Soluble dietary fibers (SDF) such as inulin are the extensively studied prebiotic fiber types in fish nutrition (Mo et al., 2015). There was only a limited number of research done on the direct application of insoluble dietary fiber (IDF) in fish diets. Therefore, the objective of the present study was to evaluate the potential use of insoluble dietary fiber extracted from cinnamon spent bark waste as a functional ingredient in the diet of Nile tilapia fingerlings.

METHODOLOGY

Collection and preparation of samples

Cinnamon spent bark waste was collected from the cinnamon processing plant of HDDES Extracts (Pvt) Ltd., Horana, Sri Lanka. The samples were sorted and cleaned with tap water before drying in a laboratory oven (DX 600, YAMATO Scientific Co., Ltd., Japan) at 60 °C until a constant weight was achieved. Then the samples were ground using a laboratory mill (ZM 200, YAMATO Scientific Co., Ltd., Japan) and sieved to obtain fine cinnamon spent bark waste powder. The powder sample was sealed and stored at room temperature until used for the analysis.

Analysis of composition

Proximate composition was analyzed according to AOAC International, (1995) methods. Procedures described by Van Soest *et al.* (1991) were used to determine the cell wall contents including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Based on the NDF, ADF, and ADL values hemicellulose and cellulose contents were calculated. Total dietary fiber (TDF), IDF and SDF contents were determined according to the enzymatic gravimetric method described by Prosky *et al*. (1992). Fiber digestion was carried out using

two samples (1 g each), which were suspended in phosphate buffer and were treated with α-amylase (Sigma-A3306, EC 3.2.1.1), protease (Sigma-P3910, EC 3.4.21.62), and amyloglucosidase (Sigma-A9913, EC 3.2.1.3). Optimum time, temperature, and pH conditions were maintained throughout the digestion. Then, the enzyme digest was filtered through a pre-weighed fritted crucible into a suction flask. The residue was washed with water, 95% ethanol, and acetone. The water washings and the filtrate were saved for the SDF analysis. The crucible with IDF was dried, weighed and then used for the determination of protein and ash. Soluble dietary fiber was precipitated with four 100 mL portions of 95% ethanol and filtered through a fritted crucible. After drying, the crucible with SDF was weighed. The summation of IDF and SDF was taken as TDF.

Extraction of dietary fiber

Insoluble dietary fiber was extracted using deionized water as described by Benitez *et al*. (2019). Accordingly, 1 g of the sample was mixed with 40 mL of deionized water and incubated at 60 °C for 60 min in a water bath (BW100, Yamato Scientific Co., Ltd., Japan). Then, it was further incubated for 30 min at 100 °C. Finally, the water-insoluble residue obtained through filtration was dried in a drying oven at 60 °C until a constant weight was achieved.

Feed preparation

The extracted IDF was mixed with a commercial fish feed powder (150–200 µm). The commercial feed (Maram Freshwater Starter Feed, Al Qassim 52761, *Saudi Arabia*) contained 44% of crude protein, 7% of crude fat, and 2% of crude fiber (Energy: 19.833 kJ/ g). Four isocaloric and isonitrogenous experimental diets were prepared by replacing the commercial feed with IDF as shown in Table 1. Gelatin (2%) was added as a binding agent, and water was added at a rate of 80 mL/ 100 g to each preparation. The content was mixed thoroughly in a mixer (MX-151SG1, Panasonic Co., Ltd, China), sun-dried and crumbles of feed were formed with an approximate size of 1 mm.

Experimental set up

All male fingerlings of *O. niloticus* with an average weight of 1.85±0.13 g were purchased from the National Inland Fisheries and Aquaculture Training Institute, National Aquaculture Development Authority of Sri Lanka. Fish were acclimatized for two weeks in cement tanks at the Department of Aquaculture and Aquatic Resources Management, University College of Anuradhapura, University of Vocational Technology, Sri Lanka. During this period, fish were fed with the commercially available powder fish feed. Twelve glass tanks (3 replicates for each treatment) with a size of 60×30×30 cm were used for the study. Eighteen fish were randomly assigned into each tank after recording initial weights. The water level was maintained at a height of 20 cm of each tank which was continuously aerated. Fish waste was siphoned out and one-third of the water was replaced with new water in each tank daily. Throughout the experimental period, fish in each tank were fed 3% of the average body weight twice a day. Fish were weighed biweekly and the amount of feed given was adjusted accordingly. The water temperature, dissolved oxygen content, salinity, and pH were recorded daily using a waterproof portable meter (CyberScan Series 600, Eutech Instruments, Singapore). Dead fish were recorded and removed daily.

Table 1: IDF contents in experimental diets

Experimental diet	IDF content (%)
Control ©	0
Treatment 1 (T1)	0.5
Treatment 2 (T2)	1
Treatment 3 (T3)	1.5

Analysis of growth performance and survival rate

At the end of 12 weeks, fish weights were recorded. The weight gain, specific growth rate (SGR), feed conversion ratio (FCR), Fulton's condition factor, and survival rate were calculated as follows.

Weight gain = (W_2-W_1) …….. Eq 1

Specific growth rate = 100 ln (W_2-W_1)/ T …… Eq 2

Where, W1, W2, and T are initial weight, final weight, and days of experimental period, respectively.

Feed conversion ratio = Feed intake/ Weight gain …….. Eq 3

Fulton's condition factor = 100 [Fish weight (g)/ Total length of fish (cm) ^{3}] …….. Eq 4

Survival rate = 100 (Number of fish survived/ Total number of fish stocked) ……. Eq 5

Microbiological analysis

At the end of 12 weeks, fish were starved for 19 h and feces were collected aseptically from each tank just after defecation. Total viable and coliform counts were determined according to Xiong *et al.* (2020) and Thunberg *et al*. (2002), respectively. Accordingly, 1 g of fecal sample was immediately transferred to 9 mL of sterilized buffered peptone water. After proper mixing, from each sample 1 mL was diluted 10-fold in sterilized peptone water. For the total viable count and coliform count, 0.1 mL from the dilution was plated on nutrient agar (Oxoid, CM0003B-500G) and MacConkey agar (HIMEDIA, MH081-500G), respectively. Then, the counts were taken after aerobic incubation at 37 °C for the duration of 24 and 48 h for coliform and total viable counts, respectively.

Blood collection and hematological analysis

After 12 weeks, selected fish from each tank were anesthetized using eugenol (0.1 mL/L). Blood collected using a hypodermic syringe from the caudal vein of the fish was treated with K_2EDTA (dipotassium ethylenediaminetetraacetic acid, 1.5 mg/mL) to prevent coagulation. The red blood cell count (RBC) and white blood cell count (WBC) were determined using the Neubauer-improved hemocytometer (Paul Marienfeld GmbH & Co. KG, Germany) as described by Lugowska et al., (2017).

Data analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 software. Results were expressed as Mean±SD. Treatment means were compared using Tukey's multiple comparison test at a 95% confidence interval.

RESULTS AND DISCUSSION

Composition of the cinnamon bark waste

Tables 2 and 3 show the proximate composition, cell wall contents, and dietary fiber contents of the cinnamon bark waste. Rabeiro-santos *et al.* (2017) reported that the ash and protein contents of *C. zeylanicum* bark were 3.77% and 4.99%, respectively. Those values are comparable to the results of the present study. However, the fat content of the cinnamon bark reported by Ribeiro-Santos *et al*. (2017) was 4.9% which was much lower in the spent bark waste after the oil extraction. Results also showed that the SDF content was very low compared to the IDF content in the cinnamon spent bark waste. According to a compositional analysis of cinnamon bark powder by Boriy, (2019), crude fiber, IDF, and SDF contents reported were 25.70%, 34.58% and 10.80% respectively, whereas Rabeiro-santos *et al.* (2017) observed 21.27% crude fiber content in cinnamon bark. The crude fiber in plants originates from certain structural units such as cellular walls and also from those become thicker when plants get mature (Śmiechowska and Dmowski, 2006). The cinnamon bark waste is composed of the outermost layers of the bark. Therefore, it carries high crude fiber and IDF levels, and low SDF content. Moreover, compared to cellulose and hemicellulose contents, cinnamon bark waste has a high amount of lignin. The

Table 2: Proximate composition and energy content of cinnamon bark waste

Component	Value
Dry matter (%)	89.57±0.01
Crude fiber (%)	43.29±0.21
Crude protein (%)	5.10±0.10
Crude fat (%)	1.57±0.15
Ash (%)	4.02±0.12
NFE* (%)	35.60±0.30
Energy (KJ/g)	19.64±0.28

Values are expressed as mean±standard deviation (n=3) on dry matter basis. (NFE*- Nitrogen-free extract)

Table 3: Cell wall and dietary fiber contents of the cinnamon bark waste

Component	Value
Neutral detergent fiber (NDF) (%)	91.45±0.05
Acid detergent fiber (ADF) (%)	84.18±1.10
Acid detergent lignin (ADL) (%)	62.87±1.01
Hemicellulose (%)	7.27±1.15
Cellulose (%)	21.31±0.52
Soluble dietary fiber (SDF) (%)	0.84±0.14
Insoluble dietary fiber (IDF) (%)	78.86±0.10
Total dietary fiber (TDF) (%)	79.70±0.86

Values are expressed as mean±standard deviation (n=3) on a dry matter basis.

cellulose, hemicellulose, and lignin contents in various sources vary depending on the type of biomass (Dhyani and Bhaskar, 2019). As Sarkanen and Ludwig, (1971) showed, lignin is very low in young shoots and high in bark . In addition, lignin has higher energy content than cellulose or hemicelluloses (Novaes et al., 2010). Therefore, it can be speculated that the high energy content of cinnamon spent bark waste may be due to the high lignin content.

Growth performance

Overall weight gain, SGR, FCR, Fulton's condition factor, and survival rates of the experimental groups are shown in the Table 4. Overall weight gain, SGR, and FCR were numerically better in T1 than other experimental groups. However, the values were not significantly different ($p>0.05$). Figure 1 shows the average weight gain of fish in experimental groups from week 2 to 12. At week 8, the average weight gain of fish in T1 was significantly higher ($p<0.05$) than the control. Average weight gain of fish in experimental groups was not significantly different in other weeks. Therefore, supplementation of IDF did not show a significant effect on the growth performance of Nile Tilapia. However, the results also showed that IDF supplementation has not adversely affected the growth performances of Nile Tilapia. Previous researches suggested that inclusion of dietary fiber in low levels is beneficial in fish farming (Li et al., 2012; Zhong et al., 2020) as it facilitates the passage of digesta at an optimal rate through the gastrointestinal tract allowing increased nutrient digestion and absorption (Li et al., 2012). Amirkolaie *et al*. (2005) assessed the effect of the type of dietary fiber on the growth of Nile tilapia where cellulose and guar gum were fed separately and as combination, at a rate of 8%. According to their results, cellulose inclusion did not show any effect on the growth while guar gum which is a type of SDF reduced the growth. Another study reported a decrease in weight gain and feed efficiency in Nile tilapia when the diet was supplemented with 10% cellulose compared to a basal diet. Moreover, 2.5 – 5% cellulose supplementation of a fish-meal based diet has increased the weight gain of juvenile Mossambique tilapia compared to a diet without cellulose. Then, the researchers concluded that the level of dietary fiber in the fish diet is important as excessive

inclusion can reduce nutrient digestibility and fish growth (Li et al., 2012). According to Ahmad *et al*. (2011), cinnamon works as a natural growth promoter in the diets of Nile Tilapia. Their study showed that supplementation of cinnamon by 1% improved the body weight gain and specific growth rate compared to the control and diets supplemented at 0.5 and 1.5% levels. Moreover, the same diet showed the lowest FCR. However, the same authors referred a previous study where, the highest growth performance and feed utilization were recorded when the diet was supplemented with 0.5% of cinnamon (Ahmad *et al.* , 2011). In the present study also the supplementation of insoluble fiber derived from cinnamon bark waste has resulted in numerically the highest growth rate and lowest FCR at the level of 1%, but those were not significantly different compared to other treatments. Comparable findings were reported in a study where European sea bass was fed with high-fiber diets obtained through the inclusion of sunflower and soybean hulls (Bonvini et al., 2018). In that study, a positive effect was not observed in weight gain, SGR, feed intake, and FCR but NDF levels varied from 7.2-15.5% (Bonvini et al., 2018). In contrast, when channel catfish was fed diets with corn bran dietary fiber, weight gain was increased with increasing dietary fiber to some extent and then decreased. The maximum weight gain was reported when the total dietary fiber level of the diet was 24.3% (Li et al., 2012). Fulton's condition factor and survival rates of the experimental groups were also not significantly different ($p>0.05$). The condition factor reflects the physiological state of fish related to welfare. It can be affected by factors such as feed, water quality, season, sex, and stress. The condition factor above one indicates isometric growth and good health conditions in fish (Migiro et al., 2014). Results of the present study showed that dietary fiber supplementation did not affect the physiological state of the fish thus, supplementation is safe which may have other beneficial effects like enhanced gut health and immunity.

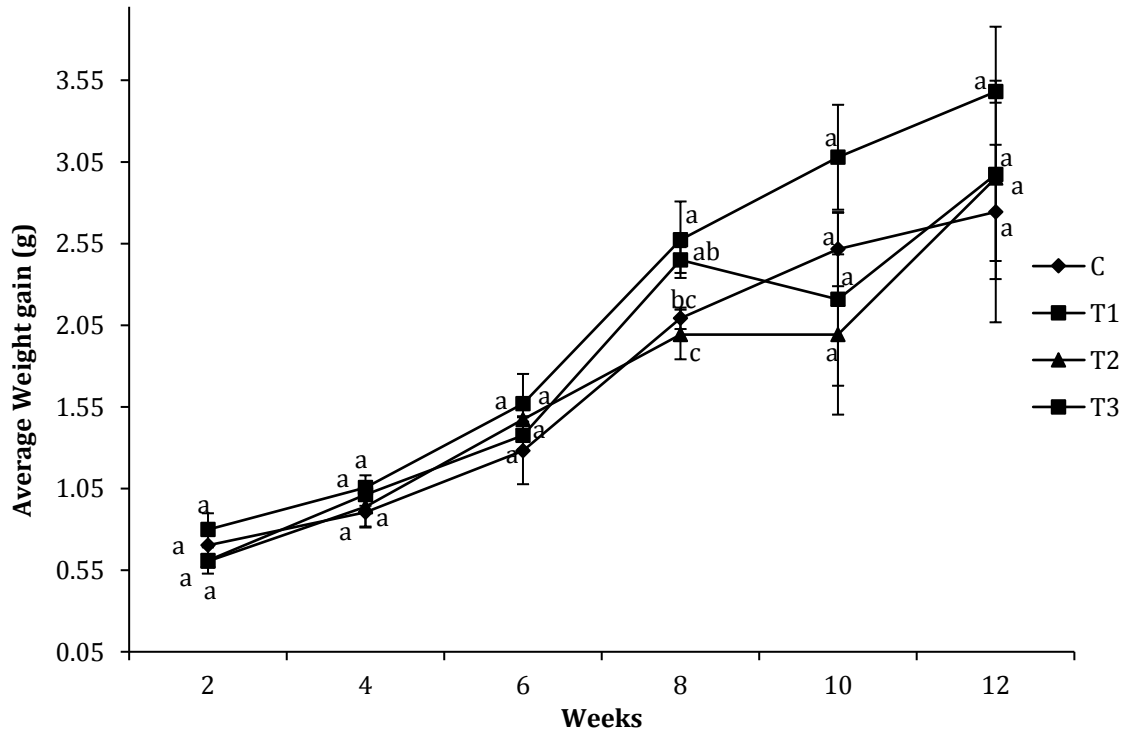

Figure 1: Mean weight gain *of O. niloticus* in experimental groups with time.
Vertical bars represent standard deviation. Means with different letters differ significantly between experimental groups at a given time point at $p<0.05$.

Table 4: Growth performance and survival rate of fish in experimental groups

Parameter	C	T1	T2	T3
Weight gain (g)	10.24±0.69[a]	12.55±0.19[a]	9.91±1.13[a]	10.63±1.50[a]
Specific growth rate (SGR) (%/day)	2.77±0.08[a]	3.01±0.02[a]	2.72±0.13[a]	2.80±0.17[a]
Feed conversion ratio (FCR)	1.29±0.06[a]	1.10±0.03[a]	1.24±0.09[a]	1.25±0.13[a]
Fulton's condition factor	1.36±0.05[a]	1.29±0.03[a]	1.36±0.09[a]	1.33±0.04[a]
Survival rate (%)	92.59±2.62[a]	90.74±2.62[a]	94.44±0.00[a]	92.59±2.62[a]

Results are expressed as mean± standard deviation (n=3). Values with different superscripts within a row are significantly different at P <0.05.

Total viable and coliform counts in feces

The total viable and coliform counts in the feces of experimental groups are shown in Figure 2. Insoluble dietary fiber supplementation at 1.5% has significantly increased ($p<0.05$) the total viable count in feces compared to the control. Moreover, IDF supplementation at 1% (T2) has significantly increased ($p<0.05$) the total viable count in feces compared to IDF supplementation at 0.5% (T1). However, the total viable counts in feces in T1 and T2 were not significantly different compared to the control. Supplementation of IDF at 0.5% and 1% have significantly decreased ($p<0.05$) the coliform counts in feces compared to those in the control group. The coliform count in feces of fish fed IDF at 1.5% was not significantly different compared to the control. Therefore, the decrease in coliform counts in feces has occurred irrespective to the level of IDF level in the diet. Prebiotics are defined as indigestible food ingredients which promote the host health through selective modification of gut microbiota (Zhou et al., 2010; Tiengtam et al., 2015; Mirghaed et al., 2018; Baenas et al., 2020). The definition of prebiotics has been broadened beyond carbohydrates to include any food component which can modify the composition/ activity of gut microbiota to improve the host health. Therefore, it also includes food ingredients such as polyphenolic compounds (Baenas et al., 2020). In general, inclusion of fiber in the diet helps to make desirable changes in the bacteria composition (Lin et al., 2020). The effects of supplementation of SDF on gut probiotics of fish have been investigated by many researchers. Soluble fibers such as inulin are known to have health benefits as they facilitate the growth of beneficial bacteria like *bifidobacteria* and *lactobacilli* in the gut. In addition, supplementing the diet of common carp fry with fructooligosaccharides was found to increase the number of lactic acid bacteria in their intestines (Tiengtam et al., 2015). Furthermore, Lin *et al.* (2020) reported that dietary fiber supplementation reduced the population of Fusobacteria and enhanced the populations of Proteobacteria and Firmicutes in the gut of Largemouth bass. Compared to SDF, the gel formation ability of IDF is low thus, fermentation of IDF by gut microbiota is limited (Amirkolaie et al., 2005). However, a recent study found that, the prebiotic activity of SDF and IDF obtained from Raspberry were not significantly different (Baenas et al., 2020). Justifying the results Baenas *et al*. (2020) explained that the unexpected prebiotic effect of IDF observed could be due to the phenolic compounds associated with cellulose in the IDF. Since the fecal microbiome represents the gut microbiome (Anslan et al., 2021) fresh feces samples were used in the present study to represent gut microbiome. Thus, the prebiotic-like effect of IDF in the gut of *O. niloticus* fingerlings observed in the present study could be due to the polyphenols attached to the IDF. This could be further justified according to Ribeiro-Santos *et al*.(2017) who reported cinnamon as a rich source of polyphenolic compounds such as catechin. However, further studies are needed to validate whether the IDF supplementation has specific benefits on the desirable gut bacteria populations.

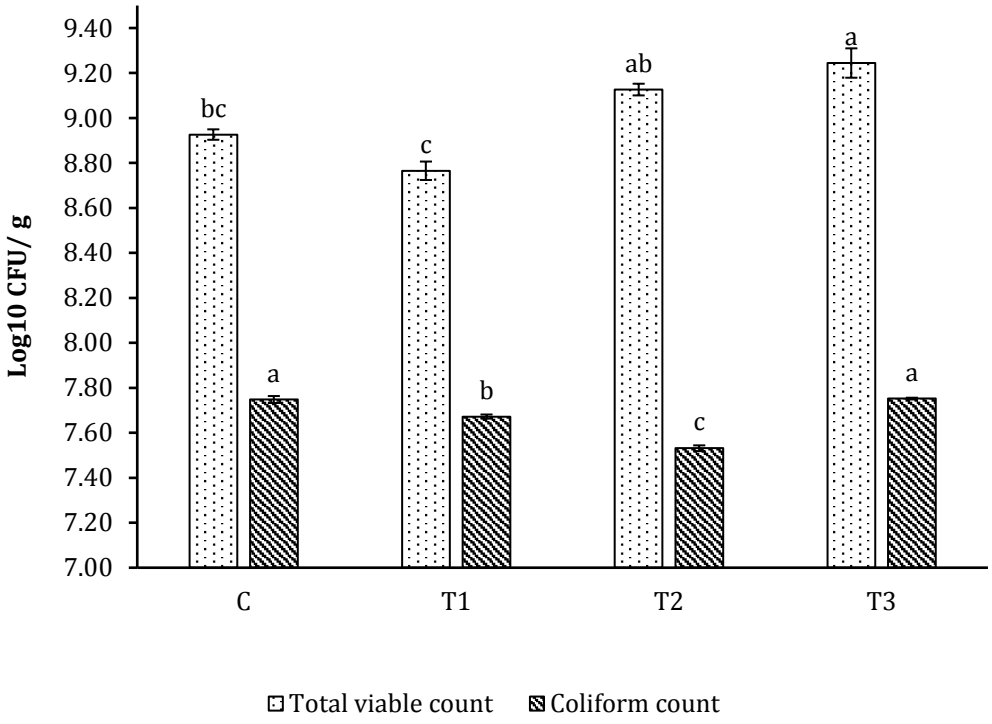

Figure 2: Total viable and coliform counts in feces of *O. niloticus* in experimental groups. Bars denoted with different letters for a given category of organisms differ significantly at $p<0.05$.

Blood parameters

Hematological parameters are widely used as indicators to assess nutritional and physiological status of fish (Bonvini et al., 2018; Hassaan et al., 2018; Ghodrati et al., 2021), as well as prebiotic activity of aquafeeds (Safari and Sarkheil, 2018). The RBC of T1 was significantly greater ($p<0.05$) than the control group. However, the RBCs of T2 and T3 were not significantly different ($p>0.05$) compared to the control and T1 (Table 5). Therefore, the RBC in fish did not increase significantly with increasing levels of dietary fiber. This could be due to the antinutritional factors associated with IDF. According to Hassaan *et al*. (2018), antinutritional factors in plant cell walls can bind with amine group of amino acids and iron which in turn will lower their availability in the blood and reduce RBC. Higher RBC level in blood is beneficial as it increases the concentration of hemoglobin and thereby the oxygen carrying capacity in fish which is beneficial under hypoxic conditions in intensive production systems (Arsalan et al., 2016; Hassaan et al., 2018). Many recent studies also suggested that inclusion of exogenous enzymes enhanced the RBC and hemoglobin contents in commercial fish species including Nile Tilapia (Ghodrati et al., 2021). Furthermore, according to Ahmad *et al.* (2011) cinnamon supplementation increased the plasma parameters including RBC in Nile Tilapia, where the highest RBC was recorded at 1 % of cinnamon supplementation. Another reason for the higher RBC in IDF supplemented fish may be associated with increased bioavailability and absorption of iron by probiotics in the gut (Ghodrati et al., 2021). However, this mechanism is not yet fully understood. Therefore, further investigations are needed to fully explain the relationship between hematological parameters and probiotics in fish under culture conditions (Ghodrati et al., 2021). Supplementation with IDF has increased ($p<0.05$) the WBCs of fish in T1, T2 and T3 experimental groups compared to the control (Table 5). In general, the white blood cell

count indicates non-specific immune response against microbial infection or presence of foreign bodies in the blood (Arsalan et al., 2016; Ghodrati et al., 2021). According to a study done by Ghodrati *et al*. (2021), it was revealed that probiotic and exogenous enzyme supplementation decreased the WBC counts in the blood of juvenile Siberian sturgeon. Low WBC counts suggests colonization of favorable microorganisms in the gut avoiding the need of producing more leukocytes to fight against pathogens (Ghodrati *et al*. 2021). However, in the current study, significantly high WBC were recorded in the fish fed with IDF. Similar results were reported in a study where the diets of *Labeo rohita* were supplemented with flaxseeds containing high levels of insoluble fiber (Page and Deshai, 2016). In addition, Safari and Sarkheil, (2018), reported that by feeding of the stem of *Cissus quadrangularis* plant with bacterial lipopolysaccharide had increased WBC and hemoglobin levels in Asian sea bass. In the same study, increased RBC, hemoglobin, and WBC in blood was observed in *Channa striata* fed with commercial prebiotics including beta-glucan, mannan-oligosaccharide and galactooligosaccharide for 16 weeks as opposed to the basal diet (Safari and Sarkheil, 2018). Furthermore, Yarahmadi *et al*. (2014, 2016) reported that administration of a dietary fiber product containing cellulose and hemicellulose had increased WBC especially monocytes in Rainbow trout and Beluga sturgeon. Many studies revealed that manupulation of gut microbiota towards beneficial communities through dietary additives can enhance the immune response of fish (Yarahmadi et al., 2014). According to Yarahmadi *et al.* (2016) increased WBC and monocytes counts after SDF administration had indicated improved immunity and health status in fish as it in turn tend to increase the lysozyme production by monocyte cells. In the same study, significant improvements had been observed in the immune related lysozyme and TNFα genes expression and measurements of innate immune response which confirm immunomodulatory effects. As explained by Yarahmadi *et al*. (2014) and Witeska *et al*. (2022), the varying results may also be due to the environmental and biological factors affecting hematological parameters in fish such as sex, age, nutrition, stress, disease and reproductive status. Moreover, both RBC and WBC are highly variable even among individuals of the same species (Witeska et al., 2022).

CONCLUSIONS

The present study did not demonstrate favorable effects of insoluble dietary fiber supplementation on the growth performance, feed conversion or the survival of *O. niloticus* fingerlings. However, insoluble dietary fiber supplementation has affected the gut microbial composition and hematological parameters in the fish. Therefore, advanced investigations on the modifications of gut microbiology and hematological parameters are suggested to ensure the use of IDF from cinnamon bark waste as a functional ingredient in the diets of *O. niloticus*.

Table 5: RBC and WBC of fish in experimental groups

Parameter	C	T1	T2	T3
RBC ($\times 10^6$ / mm^3)	1.68 ± 0.20^{a}	3.54 ± 0.82^{b}	3.06 ± 0.20^{ab}	3.02 ± 0.25^{ab}
WBC ($\times 10^5$ / mm^3)	1.53 ± 0.37^{a}	3.66 ± 0.40^{b}	3.39 ± 0.63^{b}	3.48 ± 0.71^{b}

Values are expressed as mean± standard deviation (n=3). Values with different superscripts within a row are significantly different at $p < 0.05$.

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