

Effects of Cinnamon supplemented diet on growth performance, hematological parameters, blood biochemical and immunological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings

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Abstract Dietary cinnamon supplementation has several bioactive compounds with growth-promoting and immunomodulation potential, which is suggested for finfish species. This study evaluated the inclusion of cinnamon at 0, 1, 3, 6, and 12 g/kg diet in *Oncorhynchus mykiss* fingerlings. After eight weeks, the best growth performance, and feed utilization parameters were calculated in fish treated with 12 g/kg ($P < 0.05$). Further, the white blood cells and lymphocyte levels were significantly increased and Neutrophil was significantly decreased in fish fed cinnamon at 12 g/kg compared to those fed 0-6 g/kg ($P < 0.05$). After the feeding trial, in the studied immune and biochemical indices, immunoglobulin ROS, Lysozyme was significantly increased ($P < 0.05$), and Glucose decreased significantly ($P < 0.05$) in fish treated with cinnamon at 12 g/kg compared to fish fed 0-6 g/kg. Moreover, treated fish with cinnamon had higher levels of C3, C4, total protein and Albumin than the control with the highest value in fish treated with 12 g/kg. The results showed the positive influence of the inclusion of cinnamon in the diets for rainbow trout fingerlings on the growth performance, feed utilization, blood analysis, and immune functions. According to the results, it can be concluded that 12 g cinnamon powder per kg diet is suggested with no adverse effects to improve growth performance, feed utilization and health status in rainbow trout fingerlings.

Keywords Sustainable aquaculture . Feed additives . Health condition . Hematology indices

Introduction

Increasing fish mortality is one of the most critical problems for aquaculture sector, especially in early life stages. Therefore, immune system enhancement in early life stages is one of the main keys to aquaculture success (Magnadottir 2006). It was reported that various environmental stressors can negatively affect the immune systems of fishes, which subsequently leads to different types of diseases in the aquaculture industry. These diseases can be considered the main challenge to fish breeding success, which possibly limits the economic development of aquaculture (Farahani 2011). Despite chemicals positive impacts in

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controlling, preventing, and eliminating pathogens in aquatic animals, unwanted mortalities and serious problems have been occurred by using them in aquaculture (Yao et al. 2011). Also, using antibiotics to treat and/or increase immunity in aquatic animals resulted in numerous limitations, e.g., bacterial resistance and the emergence of a new generation of bacteria, destruction of the water microbiota, administration problems, drug resistance in fish and humans and high prices. Which led to replacing them with less harmful and cheaper materials by fish farmers (Mohsenzadeh et al. 2003). There is a significant relationship between increasing disease resistance and standard feeding (Asadi et al. 2012). Also, immunostimulants can prevent the occurrence of infectious diseases by promoting the innate immune system (Watanuki et al. 2006). Therefore, nutritionists have been focused on using natural additives to replace nutritional antibiotics in recent years (Kesbiç and Yigit 2019; Kesbiç et al. 2020).

Herbal immunostimulants have been recently considered due to various features, such as stimulating the non-specific immune system (Amar et al. 2004; Rao et al. 2006), increasing tolerance to environmental stresses (Rao et al. 2006), decreasing losses due to virus, bacteria, and parasitic agents (Dugenci et al. 2003), reducing environment and aquaculture risks, improving growth indices (Adedeji et al. 2008), accelerating food absorption, enhancing therapeutic impacts, decreasing side effects, easier access, and cheaper prices (Amar et al. 2004; Rao et al. 2006; Dugenci et al. 2003). Herbal additives, especially medicinal and aromatic plants, have been shown various features, e.g. growth enhancement (Shalaby et al. 2006), appetite stimulation, anti-stress properties (Citarasu 2010), and disease resistance (Galina et al. 2009) in aquaculture. As such, many plants, parts of plants and plant extracts are tested. The effects of phytoextracts on different fish species were investigated mainly in carps and trouts with numerous plant extracts such as curcumin, paprika, thyme, oregano, garlic (Georgieva et al. 2018; 2019), nutmeg (Zhelyazkov et al. 2018), *Achillea millefolium* (Koshinski 2019), *Taraxacum officinale* (Sirakov et al. 2019; Koshinski 2020), *Acorus calamus* (Velichkova et al. 2019), etc. The use of herbs provides a cheaper way towards the progress of aquaculture. The utilization of plant extracts is promising for feed industry practice not only as they are relatively cheap, but also safe both for fish and for men as ultimate consumers (Gabor et al. 2010; 2011).

The cinnamon (*Cinnamomum* sp.), contains phenolic and polyphenolic compounds with antioxidant properties, such as eugenol, carionylene, cineol, and cinnamaldehyde (Gheibi 2005). Meanwhile, the eugenol combination has non-toxic and protective properties against various pathogens (Kunkel 1978). Besides, cinnamon has probiotic properties to promote the growth of beneficial bacteria and suppress the growth of pathogenic bacteria. Cinnamon is also a good alternative for growth chemicals. Cinnamaldehydes, polyphenols, carbohydrates, flavonoids, etc., boost up the immune system of fish and act as an important antioxidant and antibiotic. Moreover, the use of cinnamon as a growth and immunity promotor is cheap and environmentally friendly compared to other synthetic antibiotics. The effect of cinnamon on growing (Dedi et al. 2016) and fattened carps (Stoyanova et al. 2018) are limited. Salaby et al. (2006) reported the inhibitory and positive effects of cinnamon oil on fungal infections in rainbow trout. In addition, extracts of cinnamon and clove were used in some studies to exhibit potent inhibition of protein glycation and activities of anti-atherosclerotic and hypolipidemic in Zebrafish (Jina and Choa 2011).

Biologically active compounds in plant extracts could have either a positive or a negative impact on different fish species, so they have to be tested for the specific species. Although several studies were conducted on the effects of cinnamon on decreasing blood glucose and antibacterial properties on aquatic animals, our knowledge is limited on its effects on growth performance, blood indices, and immune system functions of farmed rainbow trout (*Oncorhynchus mykiss*). Therefore, the present study was aimed to investigate the effects of adding different levels of cinnamon powder to the diet on some blood factors and growth indices of rainbow trout fingerlings.

Materials and methods

Experimental fish

Seven hundred and twenty rainbow trout fingerlings were randomly selected with an average weight of 20 ± 5 g and stocked in 15 fiberglass tanks (with a volume of 1000 liters and a density of 50 fish per tank). During the experiment, the physical and chemical quality parameters of the experimental setup water (temperature,



dissolved oxygen, and pH) were also measured periodically every other two weeks by a HACH portable multimeter (model HQ40d). Water quality was averaged during the trial (mean \pm SE): water temperature, 14 ± 1.0 °C; dissolved oxygen, 7.42 ± 0.02 mg/L; pH, 7.49 ± 0.4 .

Experimental diets

Experimental diets containing five levels of cinnamon powder, as 0 (control), 1, 3, 6, and 12 g cinnamon per kg feed were used in three replications. The studied fish were daily fed 1.5 % of their body weight (Farzanfar 2007), four times a day (at 7, 12, 16, and 20 o'clock), for eight weeks. The “Faradaneh” commercial trout pellet (crude protein $44 \pm 0.02\%$ and crude lipids $15 \pm 0.05\%$), manufactured by Faradaneh Co., Iran, was used as the basic diet. Through feed lubrication with sunflower oil (50 ml/kg feed), the feed of treated trout were supplemented with 1, 3, 6, and 12 g/kg powdered cinnamon, while the control group received only sunflower oil-lubricated feed.

Growth performance determination

As growth measurements were done every two weeks, the feeding rate was adjusted accordingly. At the end of the experiment the following production parameters were determined: weight gain (WG) was calculated as (final mean weight - initial mean weight); specific growth rate (SGR) (% day⁻¹) as $100 \times [\ln(\text{final mean weight}) - \ln(\text{initial mean weight})] / \text{days}$; feed conversion ratio (FCR) as dry feed intake / wet weight gain; the condition factor (CF) was calculated as $100 \times (\text{live weight, g}) / (\text{body length, cm})^3$; standard length increments (SLI): final standard length (cm) – initial standard length (cm); daily growth rate (DGR): $100 \times [(\text{final mean weight} - \text{initial mean weight}) (\text{day}^{-1})]$; and survival rate (SR): $100 \times [(\text{Stocked fish number} \times \text{Survived fish number}) (\text{Stocked fish number}^{-1})]$ (Bulut et al. 2014). To measure the mentioned indices, samples were anesthetized with clove powder at a concentration of 300 mg/l.

Hematological and blood biochemical analysis

To hematological analysis, blood was obtained from the caudal vein using heparinized syringes (0.1 mL). White blood cells (WBC), red blood cells (RBC), hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) indices were evaluated according to Feldman et al. (2000). Furthermore, differentiated leukocytes count (monocytes, lymphocytes, and neutrophils) were performed according to Borges et al. (2004). Also, non-heparinized syringes were used to collect blood for serum separation. The samples were left for 4 h at 4 °C, then centrifuged at $3000 \times g$ for 15 min at 4 °C. Then serum samples were collected and kept at -80 °C for further biochemical analysis. Glucose (GLU), total protein (TP), albumin (ALB), C3, C4, concentrations were determined utilizing Alpha-6 Chemistry Auto Analyzer and Pars Azmon kits. To investigate the immune parameters, lysozyme enzyme and immunoglobulin M (IgM) content were investigated according to Ellis (1990) and Yamamoto and Yonemasu (1999), respectively.

Statistical analysis

The Kolmogorov-Smirnov test was employed to determine the normality of data. Statistical analysis was performed using SPSS (version 20). Mean, standard deviation and t-test were performed. The results were subjected to one analysis of variance (ANOVA) to test the effect of treatments on fish performance. Differences between means were compared using Duncan multiple range tests to test the significance level between means of treatments. The statistical significance was determined at $P < 0.05$.

Results

Growth performance

The final weight, weight gain, increasing total length, weight gain percent, specific growth rate, and daily



growth were significantly ($P < 0.05$) enhanced by supplementing cinnamon at 12 g/kg (Table 1). On the other hand, the highest values of final total length and condition factor were observed in fish fed 12 g/kg.

Hematology indices

The highest values of RBC, hematocrit, hemoglobin, MCV, MCH, MCHC, and monocytes' percentage were observed in fish treated with cinnamon at 12 g/kg diet. There were no significant differences between the studied treatments ($P > 0.05$). On the other hand, the WBC and lymphocytes were meaningfully ($P < 0.05$) increased in fish treated with cinnamon at 12 g/kg diet. Furthermore, the highest and lowest neutrophil counts were observed in the control group and fish treated with cinnamon at 12 g/kg diet, respectively (Table 2).

Blood immune and biochemical indices

The immune and biochemical indices were positively affected by adding cinnamon to diets in comparison with the control ($P < 0.05$). The highest values of immunoglobulin, ROS, lysozyme activity, and glucose were obtained under a diet containing 12 g/kg of cinnamon powder (Table 3). Despite non-significant differences in C3, C4, total protein, and albumin, the highest values of these indices were recorded in fish treated with cinnamon at 12 g/kg diet.

Discussion

Cinnamon is a beneficial medicinal spice in the series of phytochemicals involved in promoting growth performance, digestibility, antibacterial capacity, immunity, and wellbeing of aquatic animals (Abdel-Tawwab et al. 2018). Cinnamon has several bioactive compounds such as vitamins, minerals, phenolic compounds (tannins, saponins), and essential oils (cinnamic aldehyde and cinnamyl aldehyde) (Heshmati et al. 2021; Gruenwald et al. 2010) with high antibacterial capacity and antioxidative potential (Mehdipour et al. 2018). Growth and survival rates, as well as immunity, can be improved by the addition of medicinal plants to fish feed. The Flavour and storage ability of feed is also improved with added spices. Cinnamon leaf extract, cinnamon bark oil and cinnamon powder is known for significant increase in energy utilization, PER, SGR, APU, FCR, PER, FER and protein retention in fish. Cinnamon exhibits various biological activities i.e. antioxidant, antiallergic, antidiabetic and antimicrobial (Begum et al. 2018). This results in the growth reduction of harmful bacteria, which negatively affect digestion and the local intestinal immunity. The results elucidated that rainbow trout fingerlings treated with cinnamon had marked enhancement in the growth performance. The results are concurrent with several studies that investigated the growth-promoting role of cinnamon in finfish species. Obtained results revealed that adding cinnamon powder to the diet of rainbow trout fingerlings resulted in notable increases in the final weight, final length, WG, SGR, and daily growth compared to the control. Kesbiç (2019a) revealed that dietary cinnamon oil at 4 mL/kg has positive effects on the growth performance of rainbow trout. Further, rainbow trout fed dietary cinnamon at 30 g/kg led to enhanced growth performance (Ravardshiri et al. 2021). In Nile tilapia, *Oreochromis niloticus*, Rattanachaiakunsopon and Phunkhachorn (2010) reported that incorporating 0.4% cinnamon oil in diet, enhanced growth indices during a 28-day trial. In the same fish species, Ahmad et al. (2011) illustrated that adding 1% cinnamon powder in diet improved the gastrointestinal tract, secretion of digestive enzymes, excretion of toxins, and specific growth rate. Mooraki et al. (2013) reported that the dietary 1% cinnamon powder resulting non-significant increases in short-term on growth indices in Green Terror, *Andinocara rivulatus*, but specific growth rate increased significantly which may cause notable increases in other growth indices in long-term plans. Our findings showed that the lowest FCR was obtained in fish fed cinnamon at 12 g/kg, indicating high feed utilization. Similarly, Zhou et al. (2020) reported that grass carp treated with cinnamaldehyde had enhanced feed digestibility associated with high digestive enzyme activities. Also, some studies had proved the positive effects of cinnamon powder on FCR in Green Terror (Mooraki et al. 2013) and *Oreochromis niloticus* (Metwally 2009; Ahmad et al. 2011). They emphasized that cinnamon improves gastrointestinal functions and reduces the FCR. The cinnamon powder seems to increase digestion by secreting digestive enzymes caused by intestinal mucus stimulation and its antioxidant properties can



Table 1 Growth indices in rainbow trout fingerlings fed dietary cinnamon (mean \pm SD)

Item	Cinnamon (g/kg)					Sig.
	0	1	3	6	12	
n	30	30	30	30	30	
Initial weight (g)	21.27 \pm 0.16	21.22 \pm 0.43	21.25 \pm 0.53	21 \pm 0.5	21.20 \pm 0.13	-
Initial body length(cm)	11.42 \pm 95	11.50 \pm 0.28	11.80 \pm 0.33	11 \pm 0.4	11.33 \pm 0.3	-
Final weight (g)	53.65 \pm 1.63 ^b	54.20 \pm 1.3 ^b	65.70 \pm 1.03 ^{ab}	70.5 \pm 0.9 ^{ab}	74.90 \pm 0.83 ^a	0.05*
Final body length (cm)	20 \pm 0.2 ^a	20.10 \pm 0.28 ^a	21.80 \pm 0.33 ^a	22 \pm 0.4 ^a	23.21 \pm 0.15 ^a	0.62ns
Weight gain (g)	32.38 \pm 0.2 ^b	32.98 \pm 0.14 ^b	44.45 \pm 0.13 ^{ab}	49.5 \pm 0.15 ^{ab}	53.7 \pm 0.16 ^a	0.02*
Increased length (cm)	8.58 \pm 0.1 ^b	8.6 \pm 0.18 ^b	10 \pm 0.3 ^{ab}	11 \pm 0.2 ^a	12.01 \pm 0.1 ^{ab}	0.03*
RGR	152.23 \pm 18.21 ^b	155.41 \pm 19.14 ^b	209.17 \pm 9.31 ^{ab}	235.71 \pm 9.64 ^{ab}	253.30 \pm 8.29 ^a	0.05*
SGR	0.8 \pm 0.04 ^b	1.1 \pm 0.05 ^{ab}	1.80 \pm 0.06 ^{ab}	1.85 \pm 0.07 ^{ab}	1.90 \pm 0.09 ^a	0.006**
Daily growth	0.539 \pm 0.06 ^b	0.549 \pm 0.01 ^b	0.74 \pm 0.09 ^{ab}	0.825 \pm 0.02 ^{ab}	0.895 \pm 0.07 ^a	0.05*
FCR	1.8 \pm 0.10 ^b	1.5 \pm 0.19 ^{ab}	1.30 \pm 0.25 ^{ab}	1.20 \pm 0.15 ^{ab}	1.1 \pm 0.7 ^a	0.05*
Condition factor	0.67 \pm 0.16 ^a	0.66 \pm 0.19 ^a	0.63 \pm 0.25 ^{ab}	0.66 \pm 0.15 ^a	0.68 \pm 0.17 ^a	0.32ns
Survival rate (%)	98 ^a	98 ^a	99 ^a	99 ^a	100 ^a	0.22ns

Values in the same row with different letters are significantly different ($P < 0.05$). Sig. significant level; ns: non-significant and * significant at $P < 0.05$. n = the number of tested samples.

Table 2 Hematological parameters in rainbow trout fingerlings fed dietary cinnamon (mean \pm SD)

Item	Cinnamon (g/kg)					Sig.
	0	1	3	6	12	
n	15	15	15	15	15	
RBC (10^6 /ml)	2.80 \pm 0.1 ^a	2.88 \pm 0.5 ^a	2.90 \pm 0.4 ^a	2.99 \pm 0.3 ^a	3.09 \pm 0.6 ^a	0.34ns
WBC (10^3 /m)	1.53 \pm 0.03 ^b	1.55 \pm 0.04 ^{ab}	1.59 \pm 0.09 ^{ab}	1.65 \pm 0.3 ^{ab}	1.70 \pm 0.2 ^a	0.05*
HCT (%)	33 \pm 3.5 ^a	33.50 \pm 2.52 ^a	34 \pm 2.70 ^a	35 \pm 3.75 ^{ab}	36.5 \pm 4.1 ^a	0.75ns
Hb (gr/dl)	10.98 \pm 1.5 ^a	11.02 \pm 1.2 ^a	11.80 \pm 1.7 ^a	12.05 \pm 1.9 ^a	12.98 \pm 2.1 ^a	0.35ns
MCV (fl)	117.85 \pm 8.1 ^a	116.31 \pm 7.11 ^a	117.24 \pm 8.1 ^a	117.05 \pm 9.21 ^a	118.12 \pm 12.11 ^a	0.12ns
MCH (pg)	39.21 \pm 3.9 ^a	38.26 \pm 2.9 ^a	40.68 \pm 3.8 ^a	40.30 \pm 3.4 ^a	42 \pm 4.2 ^a	0.32ns
MCHC (gr/dl)	33.27 \pm 0.24 ^a	32.89 \pm 0.23 ^a	34.7 \pm 0.11 ^a	34.42 \pm 0.3 ^a	35.56 \pm 0.2 ^a	0.14ns
Neutrophil (%)	66.16 \pm 0.1 ^a	63.55 \pm 0.5 ^{ab}	53.22 \pm 0.2 ^{ab}	51.44 \pm 0.3 ^{ab}	49.59 \pm 0.4 ^b	0.04*
Monocyte (%)	2.11 \pm 0.21 ^a	1.5 \pm 0.32 ^a	1.75 \pm 0.12 ^a	1.99 \pm 0.23 ^a	2.02 \pm 0.34 ^a	0.27ns
Lymphocytes (%)	37.69 \pm 6.2 ^b	40.6 \pm 4.5 ^b	70.4 \pm 3.4 ^{ab}	73.56 \pm 4.11 ^{ab}	82.2 \pm 5.42 ^a	0.03*

Values in the same row with different letters are significantly different ($P < 0.05$). Sig. significant level; ns: non-significant and * significant at $P < 0.05$. n = the number of tested samples.

Table 3 Blood immune and biochemical indices in rainbow trout fingerlings fed dietary cinnamon (mean \pm SD)

Item	Cinnamon (g/kg)					Sig.
	0	1	3	6	12	
n	15	15	15	15	15	
Immunoglobulin mg/dL	72.23 \pm 18.25 ^b	100.23 \pm 14.23 ^{ab}	105.35 \pm 17.11 ^{ab}	111.23 \pm 16.12 ^{ab}	115.36 \pm 14.2 ^a	0.04*
C3 mg/dL	21.24 \pm 6.25 ^a	32.70 \pm 5.78 ^a	33.24 \pm 4.15 ^a	35.29 \pm 3.80 ^a	37.88 \pm 4.22 ^a	0.45ns
C4 mg/dL	6.38 \pm 2.61 ^a	8.20 \pm 2.50 ^a	8.22 \pm 2.42 ^a	8.38 \pm 2.34 ^a	9.31 \pm 2.03 ^a	0.12ns
ROS RLU _s ⁻¹	486.2 \pm 56.3 ^b	1100.44 \pm 121.33 ^{ab}	1213.6 \pm 156.85 ^{ab}	1315.66 \pm 214.25 ^{ab}	1380.25 \pm 220.12 ^a	0.03*
Lysozyme μ g/mL	2.40 \pm 0.56 ^b	5.24 \pm 0.44 ^{ab}	5.38 \pm 0.65 ^{ab}	5.65 \pm 0.47 ^{ab}	5.98 \pm 0.56 ^a	0.023*
Glucose mg/dL	176.62 \pm 14.56 ^a	174.24 \pm 8.89 ^{ab}	170.56 \pm 9.15 ^{ab}	165.64 \pm 9.31 ^{ab}	150.55 \pm 8.56 ^b	0.04*
Total protein g/dL	340 \pm 0.56 ^a	4.02 \pm 0.54 ^a	4.11 \pm 0.46 ^a	4.20 \pm 0.52 ^{ab}	4.80 \pm 0.38 ^a	0.14ns
Albumin g/dL	2.40 \pm 0.46 ^a	2.12 \pm 0.54 ^a	2.21 \pm 0.35 ^a	2.42 \pm 0.26 ^a	2.90 \pm 0.31 ^a	0.44ns

Values in the same row with different letters are significantly different ($P < 0.05$). Sig. significant level; ns: non-significant and * significant at $P < 0.05$. n = the number of tested samples.

also improve the absorption of nutrients.

The survival rate is the most important indicator that shows the general immunity condition against pathogens and environmental stressors. Our findings revealed that although the maximum possible survival rate was recorded in fish fed with cinnamon powder at 12 g/kg diet, there was no significant difference compared with other treatments and the control. In this regard, the reported results by Rattanachaiakunson and Phunkhachorn (2010) and Ahmad et al. (2011) indicated that there was no significant difference in the survival rate of Nile tilapia fed with different levels of cinnamon. However, although reported that notable changes in fish survival rates have commonly been observed for treating periods longer than six months,



proper conditions of study and water quality can lead to reducing fish losses and increasing survival rates (Jina and Choa 2011).

Hematological parameters are important in diagnosing the health and physiology of fish which are affected by nutrition conditions, environmental factors, age, sexual cycle, and other physiological parameters (Kazerani Farahani 2009). Meanwhile, WBC is one of the vital indicators for the immune system of organisms. It was clear that the percentage of neutrophils, lymphocytes, monocytes, stress, disease, pollutants, nutritional conditions, ecological conditions, age, and sex affect the number of WBC (Farahani 2011). The fishes' immune system is improved by organs producing WBC (Farahani 2011). In the present study, the highest content of WBC was calculated in fish treated with cinnamon at 12 g/kg diet, significantly different from the control ($P < 0.05$). Immune stimuli cause changes such as increasing phagocytic cells and lymphocyte and macrophage activities in fish, these components act as a non-specific fish defense system and are of particular importance in the health and immune system of fish (MacArthur and Fletcher 1985). Based on our findings, increases in stimulation and strengthening of the immune system and survival rate in rainbow trout fingerlings, as well as the decrease in infectious diseases, can be related to the WBC increasing by dietary cinnamon.

Hemoglobin and hematocrit directly depend on the changes in RBC, therefore increasing hemoglobin content has direct effects on fish respiratory gas transport, heart rate, and final weight (Kazerani Farahani 2009). In the present study, despite the non-significant increases in erythrocyte, hemoglobin, and hematocrit indices, the relative superiority of fish respiratory status in treatments containing cinnamon powder can be related to cinnamon effects on improving blood parameters. Kesbiç (2019a) showed that 4 mL cinnamon oil in a one kg diet can enhance RBC, Hb and hematocrit in rainbow trout. Dietary 1% cinnamon powder had no significant effects on hematological indices of Green terror (Roozy et al. 2013). Ahmad et al. (2011) showed that 1% cinnamon powder in diet can increase significantly the number of RBC, hemoglobin, and hematocrit in Nile tilapia. Also, Kesbiç (2019b) showed that 5-10 mL juniper berry oil in a one kg diet can enhance RBC, Hb and hematocrit in common carp (*Cyprinus carpio*). Mazandarani and Hoseini (2017) have reported, in their anaesthesia study on juvenile carps, that 1.8-cineole exhibits a dose-dependent sedative effect in fish. It is believed that the increase in the hemoglobin levels due to the consumption of herbal additives is the result of the ventilation slowing down because of sedation (Forgan and Forster 2010). Therefore, it can be concluded that immune stimulants may have no significant effects on RBC, hemoglobin, and hematocrits.

Hematological and biochemical blood indices reflect the health status of fish when treated with various feed additives (Zemheri-Navruz et al. 2019; Dawood et al. 2021). Age, feeding, sexual maturity, photoperiod, water quality, water temperature and method for blood sampling are factors influencing blood biochemical parameters in fish (Coşkun et al. 2016; Fazio et al. 2016). However, fish blood glucose level has been calculated at ranges of 25 to 350 mg/dl in normal conditions, depending on the fish species (Thrall 2004), which was estimated 150.6-176.6 mg/dl in the present study. Kesbiç (2019a) showed a decrease in serum glucose level by increasing dietary cinnamon oil level in rainbow trout. Also, more reports are available on the dietary cinnamon powder's antioxidant effects on insulin activity which shown decreasing blood glucose and increasing cell metabolism processes (Roozy et al. 2013; Gheibi et al. 2005). Broadhurst et al. (2000) reported that cinnamon polyphenolic compounds affect the rats' pancreas and lead to hypoglycemia and increasing glucose metabolism due to increased insulin secretion. Accordingly, it seems that cinnamon powder has efficient role in hypoglycemia and increasing cell metabolism due to the diverse effects of its polyphenolic compounds on insulin secretion and strengthening insulin function.

The IgM antibody changes in fish fed with cinnamon powder at 12 g/kg diet had a significant increase compared to the control. Generally, it can be concluded that antibody titer changes were concentration-dependent on the cinnamon powder. In confirming the positive effects of herbal compounds on the immunoglobulin of fish, Yin et al. (2009) showed that the extracts of *Astragalus radix* and *Ganoderma lucidum* significantly enhanced the carp immune system. Changes in serum complement are substantial in protecting the nonspecific immune system of fish so that fish health is directly associated with increased plasma C3 and C4 levels (Yano et al. 1996). The C3 and C4 slightly increased in treatments containing cinnamon powder compared to the control. It can be affected by additive plant species, compound type, various concentrations, and fish species.

Plant stimulants can significantly increase phagocytosis respiratory burst activity in fish. ROS is one



of the main specific factors involved in this process that are secreted by some phagocytic cells such as neutrophils. The results of this study showed that ROS changes in treatments containing cinnamon powder increased significantly, also enhanced with increasing concentrations of the plant composition. Yin et al. (2009) reported that indices of respiratory burst activity and ROS production increased by using *Astragalus radix* extract at 0.5% and the combination of *Astragalus radix* + *Ganoderma lucidum* extracts. Ardo et al. (2008) reported that using 0.5% of *Astragalus membranaceus* and *Lonicera japonica* extracts, individually and in combination, led to significant increases in the respiratory burst activity and ROS content. Our obtained results were in line with the results of Peddie and Secombes (2003), who reported an increase in the respiratory burst and ROS activities in trout affected by *Lavandula angustifolia*, *Eupatorium perfoliatum*, and *Baptista tinctoria* extracts.

Lysozyme is one of the components of the body's nonspecific immune system that penetrates bacterial cells by removing the 1-4 glycosidic bonds between hard components of the cell wall of gram-positive bacteria, such as peptides and glycans. Our results showed a positive significant relationship between increasing cinnamon powder levels and lysozyme activity, which was consistent with the results of Yin et al. (2009). Pourgholam et al. (2013) stated that the highest lysozyme activity was observed at a combined concentration of 1.5 g/kg *Echinacea* and 5 g/kg *Astragalus* in trout fish. Therefore, it can be concluded that there is a direct association between the concentration of plant compounds and lysozyme activity.

Conclusion

The data resulted from the present study indicate the growth performance traits and immunological indices of rainbow trout fingerlings were positively influenced by supplementation of feed with cinnamon powder. The hematological parameters showed a positive influence of cinnamon. The tested dietary supplement had no side effects on studied blood biochemical parameters. Better results in fish that received a ration supplemented with cinnamon were probably due to the high content of vitamins, minerals and phenolic compounds in the cinnamon, as well as to improved organoleptic quality of feed and its digestibility. Therefore, it can be concluded that 12 g/kg of cinnamon powder is required with no adverse effects for better performances of rainbow trout fingerlings.

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Conflicts of interest The authors declare no conflict of interest.

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