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RESEARCH PAPER

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Changes in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Growth Performance, Digestive Enzyme Activity, Hematological Profile and Serum Biochemical Markers After Dietary Administration of γ-irradiated Cinnamon (*Cinnamomum verum*) Ethanolic Extract

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Abstract

The aim of this study was to investigate the effects of dietary supplementation of γ-irradiated cinnamon ethanolic extract on the growth performance, digestive enzymes, lysozyme activity level and hematological factors in the juvenile rainbow trout. The fish were divided into five groups, including control group and four experimental groups (received diets enriched with 10 g/kg of non-irradiated or irradiated CEE at the radiation doses of 10, 20 and 40 KGy. At the end of the feeding trial (60' days), 10 fish were sampled from each tank after 12 h of feed deprivation. The results showed that the fish dietary irradiated CEE at doses of 10 KGy and 20 KGy had significantly higher final weights, weight gain, specific growth rates and growth hormone levels, and lower feed conversion ratios compared to the other groups ($P \leq 0.05$). The blood glucose level significantly lowered in the fish group fed with dietary γ-irradiated CEE, and increased the activity of lysozyme activity level and digestive enzymes compared to the control group ($P \leq 0.05$). In conclusion, dietary supplementation of γ-irradiated CEE at a dose of 10 KGy can enhance the positive effects of cinnamon extract and improve the growth performance, digestion and lysozyme activity in juvenile rainbow trout.

Introduction

Global aquaculture has grown rapidly over the past decades due to increased global demand for seafood. However, increased seafood production, intensive fish farming and ineffective management of the fisheries have increased the rates of diseases and decreased growth performance in fish by inducing undesirable changes in the environment (FAO, 2020; Finegold, 2009).

Synthetic growth promoters such as several antibiotics have been used in fish diets for decades, due to their positive effects on the growth rate, weight gain and disease prevention (Cabello, 2006). As a result, there is a need for alternative natural additives to replace synthetic growth promoters.

Currently, medicinal herbs are under research as potential candidates to replace antibiotics due to their being more cost-effective and having fewer side-effects. Cinnamon (*Cinnamomum verum*) is a popular spice that has also been used as a medicinal herb for centuries.

Its bark and leaves contain many bioactive compounds such as cinnamaldehyde, cinnamic acid, trans-cinnamaldehyde, cinnamyl alcohol, flavonoids and eugenol, and exert antimicrobial, antifungal, antioxidant, anti-inflammatory, anti-diabetic and anti-cancer effects (Gruenwald et al., 2010; Vasconcelos et al., 2018). Cinnamaldehyde and trans-cinnamaldehyde are said to be responsible for the antimicrobial properties of cinnamon (Arancibia et al., 2014; Rattanachaikunsopon and Phumkhachorn, 2010; Vasconcelos et al., 2018).

Eugenol, a phenolic compound from cinnamon oil has antifungal effects and has been able to inhibit the growth of *F. proliferatum* in maize grain (Velluti et al., 2003). Cinnamon essential oil exerted antibacterial properties against *Vibrio spp.*, *Streptococcus iniae, Listeria monocytogenes, Photobacterium phosphoreum, Aeromonas hydrophila* and *Enterococcus faecalis* (Abdel-Tawwab *et al.* 2018; Ahmad et al., 2011; Arancibia et al., 2014; Habiba et al., 2021; Huq et al., 2015; Rattanachaikunsopon and Phumkhachorn, 2010; Velluti *et al.* 2003; Ali et al., 2021). Cinnamon also has shown antiviral activity against influenza, HIV-1 and HIV-2 (Gruenwald et al., 2010).

When added to the diet of broiler chickens, cinnamon led to increased final weight and decreased feed conversion ratio (FCR) without altering the odor or flavor of the meat (Shirzadegan, 2014; Toghyani et al., 2011). In aquaculture, supplementation of cinnamon in Nile tilapia (*Oreochromis niloticus*), European sea bass (*Dicentrarchus labrax*) and Common carp (*Cyprinus carpio*) displayed positive results in growth performance, immunity and pathogen-resistance (Abdel-Tawwab et al., 2018; Habiba et al., 2021; Mohammad, 2021).

Gamma (γ)-ray is a powerful procedure for destroying harmful microorganisms and increasing the safety and shelf-life of the products in the food and pharmaceutical industry (Hassan et al., 2019). It is mentioned that γ-irradiation can enhance the antimicrobial effects of cinnamon oil (Lyu et al., 2018). It also increases the total flavonoids and phenolic compounds and decreases the generation of free radicals in cinnamon (El-niley and Farag, 2012; El-megid et al., 2018).

Since the γ-irradiation can alter the composition of cinnamon and improve its antimicrobial and antioxidant abilities, adding irradiated cinnamon extract to a fish diet may improve general health and growth performance. It could be a potential growth enhancer and may even replace antibiotic growth promoters in aquaculture. Therefore, this study was conducted to evaluate the potential effects of dietary supplementation of γ-irradiated cinnamon ethanolic extract (CEE) on growth performance, body composition, biochemical factors and lysozyme activity level in juvenile rainbow trout.

Material and Methods

Groups

A total number of 300 juvenile rainbow trout with an average body weight of 26±0.04 g were obtained from Yazdani farm, Dalkhan, Sepidan, Fars, Iran. Healthy juvenile rainbow trout were kept in an indoor tank for 10 days for acclimation to the laboratory conditions. Fish were randomly assigned to 5 experimental groups and separated into triplicate tanks (300 L) per group (n=20 per tank). The tanks were equipped with water inlet and outlet. All tanks were washed carefully, filled with water and constantly aerated. During the study, temperature, pH, and oxygen values were maintained between 15.5 and 16°C, between 7.9 and 8.2 and between 7 and 7.2 mg $L^{-1,}$ respectively. At first, the fish were acclimatized to the experimental condition for 10 days and fed the control diet three times a day (10:00, 13:00, 17:00) until satiation. Then, the fish from different groups were fed their corresponding diets three times daily (10:00, 13:00 and 17:00 h) until satiety for 60 days. Also, the mortality of fish was monitored for the period of the experiment in all the groups.

Fish were divided into five groups (a control group (without cinnamon extract), and the 4 treatment groups consisted of 3 irradiated and a non-irradiated cinnamon extract supplemented diet. The initial weight was recorded before stocking the fish into the tanks. The feeding trial lasted for 60 days, during which the treatment groups received dietary supplements of 10 g/kg of γ-irradiated cinnamon ethanolic extract. The γ-irradiation doses varied between the experiment's groups (10 KGy, 20 KGy and 40 KGy). The sampling took place at the end of the experiment and after 12 hours of feed deprivation.

Preparations of Cinnamon Ethanolic Extract (CEE)

Cinnamon bark was purchased at a spice unit in a supermarket in Shiraz. Firstly, the cinnamon bark was washed and cut into small pieces. Cinnamon bark was dried using a drying cabinet at 40 - 42°C for 3 - 4 days. The dried plant material was ground into powder using a laboratory blender, sieved with mesh to obtain the very fine powder, then stored at room temperature in a dry and sterile container. To prepare the ethanolic extract, 25 g of cinnamon powder was weighed and placed into a flask. Ethanol (250 mL) solvent with a concentration of 96% was added to the flask and the plant materials were submerged in the solvent for two weeks at room temperature with regular shaking. It was then filtered and the solvent evaporated using a rotary evaporator and then kept at 4°C (Parekh and Chanda, 2007).

γ-irradiation of the Cinnamon Ethanolic Extracts (CEE)

The cinnamon ethanolic extract (CEE) was irradiated using the γ-cell model PX-30 (Russia) at a dose rate of 0.02 Gy/S at the Nuclear Science and Technology Research Institute, Karaj, Iran. The irradiation doses of 10, 20 and 40 KGy were considered for the CEE. After the irradiation, the samples were stored at 4°C for further analysis.

Carcass Composition Analysis

Body composition analyses were carried out for protein, fat, dry matter and ash according to the Association of Official Analytical Chemicals method

(AOAC) (AOAC, 1990). Dry matter (DM) was determined by drying the samples to a constant mass at 105°C overnight and ash content was measured by igniting the samples in a muffle furnace at 525°C for 8 h. Nitrogen content (N) was measured by the Kjeldahl method (AOAC, 1990). The crude protein was calculated as N × 6.25. The fat content was determined using a solvent extractor (Behr Labor-Technik, Germany) equipped with six Soxhlet posts.

Growth Performance

The fish were weighed before and after the feeding, the trial was to determine the initial weight and final weight.

Weight gain = Initial weight – Final weight

Feed conversion ratio (FCR) = Feed intake (g)/ weight gain (g)

Specific growth rate (SGR) (%) =100 [final weight (g)/Initial weight (g)]/Experiment period.

Hematology Profile

At the end of the trial, 10 fish from each tank were separated and feed-deprived for 12 h prior to blood sampling. They were then anesthetized with clove oil bath (50 µl l^{-1}), and the blood samples were collected from the caudal peduncle using heparinized syringe.

The collected blood was then divided into two aliquots. One was heparinized, and the other one was allowed to clot for 30 min at room temperature, and the serum was separated by centrifugation at 300 rpm at 4°C after 5 h of maintenance. The heparinized blood was immediately utilized for the hematological assays.

Red blood cells (RBCs) and white blood cells (WBCs) were counted using Neubauer hemocytometer after diluting the samples with phosphate-buffered saline (PBS). Hematocrit values (Ht) were determined by collecting freshly drawn blood in capillary tubes and centrifuging for 5 min in a microhematocrit centrifuge.

Blood Biochemistry and Liver Function Enzymes

Glucose, total protein, total lipids, urea and creatinine were measured using the kits from Pars Azmun Company (Pars Azmun, Iran) according to the protocols. Serum alkaline phosphatase) (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by an autoanalyzer (Eppendorf, EPOS, and Germany).

Digestive Enzymes

Digestive tracts of the fish were collected, homogenized on ice in cold double-distilled water and centrifuged at 9000 × g for 20 min at 4°C. The supernatants were stored at - 80°C to determine the activity levels of the digestive enzymes (amylase, lipase and protease).

Protease activity was measured as previously described (Hidalgo et al., 1999), using casein (1%) hydrolysis at pH 8. Tyrosine was used as standard, and one unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1 µg of tyrosine per minute.

Lipase activity was assayed by the hydrolysis of p-Nitrophenyl myristate (Sigma-Aldrich) according to a method described by Iijima et al., (1998). Specific activity (U) was expressed as:

Lipase activity = (A (sample (280nm)) × value ×1000)/ (15 ×16500×mg protein)

Amylase activity was determined using starch (2%) as a substrate according to prior references (Bernfeld, 1955; Worthington, 1991). Specific activity (U) was expressed as:

Amylase activity = (Maltose released (µmol))/ (3×mg protein)

Serum Lysozyme Activity

Serum lysozyme activity was calculated by the turbidimetric assay as described by Demers and Bayne (1997). *Micrococcus lysodeikticus* (Sigma) suspended in 0.1 M phosphate-citrate buffer (pH 5.8) at a concentration of 750 µg ml^{-1} was added to 25 µL of serum samples in 96-well microtiter plates. The optical density was determined immediately after the addition of 150 µL of *M. lysodeikticus.* One lysozyme activity unit was defined as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1}.

Serum Growth Hormone (GH) Levels

The serum growth hormone (GH) levels (18 specimens per treatment) were measured using enzyme-linked immunosorbent assay (ELISA) according to the method (Li et al., 2010). All ELISA experiments were conducted in triplicate. The absorbance was measured at 450 nm using a microplate reader (MR4, Hiperion, Germany) and recorded using a computer Standard curve generated by plotting the absorbance against the logarithm of the analyte concentration (García-Nieto et al., 2010).

Statistical Analysis

All data are presented as means ± standard deviation (SD) of three replicates. Statistical analysis of normal distribution and variance homogeneity of the data was confirmed by Shapiro-Wilk and Levene tests, respectively. After confirming the normality of data and homogeneity of variances, the one-way analysis of

variance (ANOVA) was conducted using SPSS ver. 17.0 (IBM Corp. USA). Multiple comparisons of obtained data between treatments at each group were made using Tukey. The P value <0.05 was used as the level of statistical significance in all analyses.

Results

Growth Performance Analysis

No mortality was observed in fish of different treatments and control groups during the 60-day feeding trial. The data presented in Table 1, show that fish fed with γ-irradiated CEE supplemented diets had an increase in the final weight, weight gain and SGR, and a significant decrease in FCR to the control group. The highest increase in the SGR is shown in the group receiving CEE irradiated at a dose of 10 KGy of gamma ray (P≤0.05). The treated group exhibited the best FCR values, especially fish that received γ-irradiated CEE at doses of 10 KGy and 20 KGy, to the control group (P≤0.05). In general, these positive changes were more prominent in the groups that received irradiated CEE at doses of 10 KGy and 20 KGy, than in the 0 KGy and 40 KGy groups (P≤0.05).

Hematology Profile and Serum Lysozyme Activity Level

Table 2 demonstrates the results of RBC, Ht, HB and WBC analyses. The RBC levels were higher in the group of fish fed with 10 g/kg of CEE γ-irradiated at a dose of 20 KGy (P≥0.05). Hct (%) significantly increased in 0 KGy, 10 KGy and 20 KGy groups, while there was a decrease in the control group and group received γ-irradiated CEE at a dose of 40 KGy (P≤0.05). Finally, WBC levels increased in all treatment groups that received non-irradiated or γ-irradiated CEE, with the highest levels being observed at a dose of 20 KGy of gamma ray; however, the changes were not statistically (P≥0.05).

Table 1. Growth performance (Mean ± SE) of juvenile rainbow trout fed diets containing different dosages of γ-irradiated and non-irradiated cinnamon extract for 60 days.

Groups	Cinnamon Extract 0 g/kg	Cinnamon Extract 10 g/kg			
	Control (Without additive)	(Non-irradiated)	10 kGy (Irradiated)	20 kGy (Irradiated)	40 kGy (Irradiated)
Initial weight (g)	26.22±0.07	26.25±0.04	26.24±0.04	26.21±0.03	26.24±0.07
Final weight (g)	76.63±0.24^{c}	81.95±0.18^{b}	88.41±0.31^{a}	87.89±0.16^{a}	81.35±0.19^{b}
Weight gain (g)	50.41±0.22^{c}	55.34±0.29^{b}	61.87±0.22^{a}	61.67±0.21^{a}	55.37±0.25^{b}
FCR	1.39±0.03^{a}	1.28±0.03^{b}	1.13±0.03^{c}	1.14±0.02^{c}	1.27±0.02^{b}
SGR (%)	1.79±0.02^{c}	1.88±0.02^{c}	2.19±0.02^{a}	2.01±0.03^{b}	1.91±0.02^{d}

*Means having the same letter or no letter in the same row are not significantly different at (P≤0.05).
(n = 30 fish per treatment).

Table 2. The effects of different dosages of γ-irradiated and non-irradiated cinnamon extract fed to juvenile rainbow trout for 60 days on hematological markers; Erythrocyte count, Hematocrit, Hemoglobin and Leukocyte count.

Groups	Cinnamon Extract 0 g/kg	Cinnamon Extract 10 g/kg			
	Control (Without additive)	(Non-irradiated)	10 KGy (Irradiated)	20 KGy (Irradiated)	40 KGy (Irradiated)
RBC (×10^{12}/L)	1.58±0.15	1.55±0.21	1.61±0.21	1.66±0.22	1.60±0.15
Hematocrit (HCT) (%)	33.25±1.65^{b}	37.21±1.02^{a}	37.46±1.19^{a}	37.29±0.44^{a}	33.98±0.87^{b}
HGB g\dL	5.76±0.44	5.59±0.52	6.17±0.31	5.98±0.41	5.22±0.39
WBC (×10^{9}\/L)	19.99±1.21	21.55±1.72	22.08±1.02	22.12±1.43	21.01±1.45

*Means having the same letter or no letter in the same row are not significantly different at (P≤0.05). (n = 30 fish per treatment)

Table 3. Chemical analysis of the carcass in juvenile rainbow trout fed with non-irradiated and γ-irradiated cinnamon extract at different dosages for 60 days.

Groups	Cinnamon Extract 0 g/kg	Cinnamon Extract 10 g/kg			
	Control (Without additive)	(Non-irradiated)	10 KGy (Irradiated)	20 KGy (Irradiated)	40 KGy (Irradiated)
Crude Protein (%)	57.43±1.27	57.55±1.22	58.98±1.28	58.18±1.39	57.44±1.25
Fat (%)	23.28±0.33^{d}	24.37±0.21^{c}	26.17±0.23^{b}	27.47±0.18^{a}	26.29±0.16^{b}
Ash (%)	18.43±0.24	18.51±0.32	18.66±0.21	18.46±0.54	18.13±0.92
Dry matter (%)	34.23±2.21	34.72±1.49	35.28±1.02	35.19±1.37	34.56±1.57

*Means having the same letter or no letter in the same row are not significantly different at (P≤0.05). (n = 30 fish per treatment)

Carcass Composition Analysis

Table 3 shows the body composition analysis in the control and treatment groups. A small but non-significant increase was observed in crude protein, ash and dry matter content in all treatment groups, compared to the control group. The highest levels of crude protein, ash and dry matter were found in the group that received irradiated CEE at 10 KGy (P≥0.05). The ash content decreased again in the 40 KGy group. On the other hand, fat content faced a significant increase in treatment groups, significantly in the 20 KGy group (P≤0.05).

Blood Biochemistry and Liver Function Enzymes

Table 4 shows the results of the biochemical tests. The total protein concentration increased, mainly in the 10 KGy group; however, it was not statistically significant (P≥0.05). Serum total lipids increased meaningfully in all of the treatment groups (P≤0.05). Glucose levels also decreased significantly in all of the treatment groups (P≤0.05). The lowest glucose levels were observed in the 20 KGy group. Urea levels decreased, mainly in the 10 KGy group; however, the levels were statistically non-significant (P≥0.05). Additionally, no meaningful changes were observed in creatinine levels (P≥0.05). Serum AST and ALT activities elevated significantly, with the highest levels being observed in the 10 KGy group, while ALP levels decreased in the 10 KGy and 20 KGy groups (P≤0.05). Figure 1 showed that lysozyme activity levels increased significantly in the 10 KGy and 20 KGy treatment groups compared to the control, non-irradiated and 40 KGy treatment groups (P≤0.05).

Digestive Enzymes and Growth Hormone Level

Figure 2, 3 and 4 demonstrate the levels of amylase, protease and lipase in control and treatment groups. Amylase, protease and lipase activity levels increased significantly in 0 KGy, 10 KGy and 20 KGy treatment groups, but then decreased in the 40 KGy treatment group (P≤0.05). The highest protease activity levels were observed in the 20 KGy treatment group, while the highest lipase activity levels were observed in the 10 KGy and 20 KGy groups (P≤0.05), but was no significant difference between 10 KGy and 20 KGy groups and non-irradiated CEE treatment group in lipase activity levels (P>0.05). The lowest activity levels of protease and lipase were recorded in the control group (feeding on a diet devoid of any treatment) to the

Table 4. The effects of different dosages of γ-irradiated and non-irradiated cinnamon extract fed to juvenile rainbow trout for 60 days on biochemical markers; Total protein, Total lipids, Glucose, Blood urea, Creatinine, Serum AST, Serum ALT and Serum ALP

Groups	0 g/kg	Cinnamon Extract 10 g/kg			
	Control (Without additive)	(Non-irradiated)	10 KGy (Irradiated)	20 KGy (Irradiated)	40 KGy (Irradiated)
Total protein	37.01±3.64	40.87±4.61	41.32±4.42	41.01±5.12	38.99±3.62
Total lipids	725.6±44.16^{c}	814.3±51.33^{b}	865.3±50.12^{ab}	936.45±49.96^{a}	809.63±54.67^{b}
Glucose	77.92±2.46^{a}	59.13±2.11^{c}	55.82±2.34^{c}	49.63±1.91^{d}	62.44±2.57^{b}
Urea	12.43±0.45	12.08±0.41	11.89±0.47	12.10±0.46	12.25±0.46
Creatinine	0.48±0.03	0.49±0.05	0.47±0.04	0.48±0.04	0.51±0.03
AST	93.51±1.99^{c}	93.14±1.86^{c}	110.47±0.97^{a}	99.65±0.98^{b}	90.46±1.85^{c}
ALT	22.73±1.35^{b}	24.56±0.81^{b}	28.22±1.01^{a}	26.89±1.45^{a}	23.69±0.88^{b}
ALP	78.32±0.06^{a}	76.54±0.05^{c}	74.22±0.06^{d}	73.99±0.07^{d}	75.36±0.12^{b}

*Means having the same letter or no letter in the same row are not significantly different at (P≤0.05). (n = 30 fish per treatment)

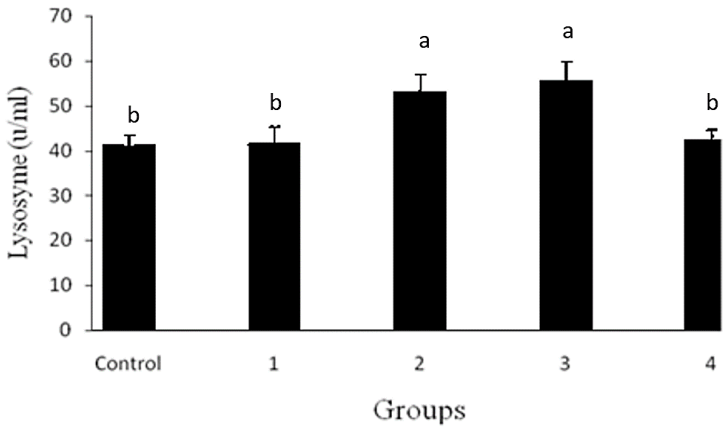

Figure 1. Evaluation of the changes in the serum concentration of lysozyme in juvenile rainbow trout (P≤0.5); (n=30 fish per treatment).

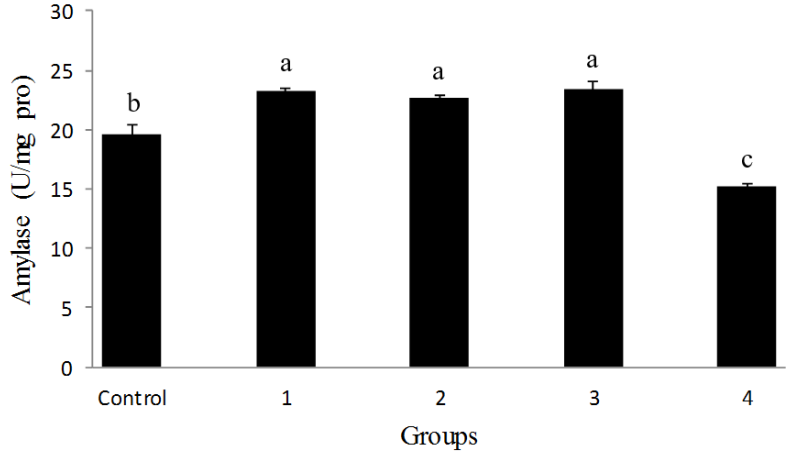

Figure 2. Evaluation of the amylase activity of the digestive tract in juvenile rainbow trout (P≤0.5); (n = 18 fish per treatment)

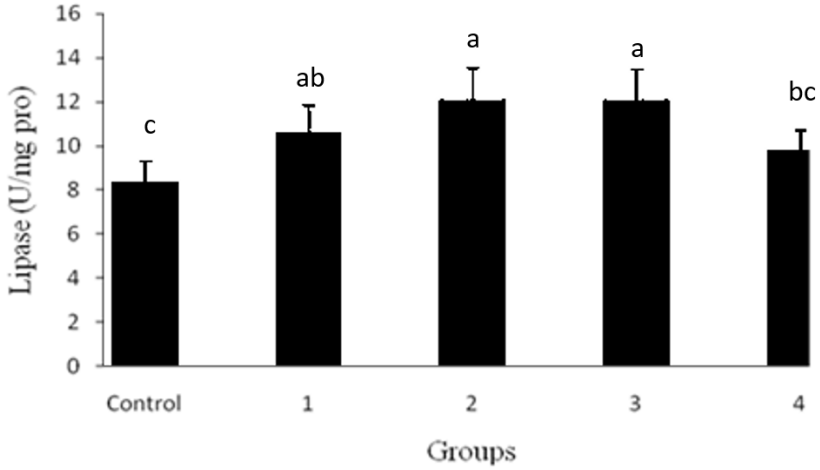

Figure 3. Evaluation of the lipase activity of the digestive tract in juvenile rainbow trout (P≤0.5); (n = 18 fish per treatment)

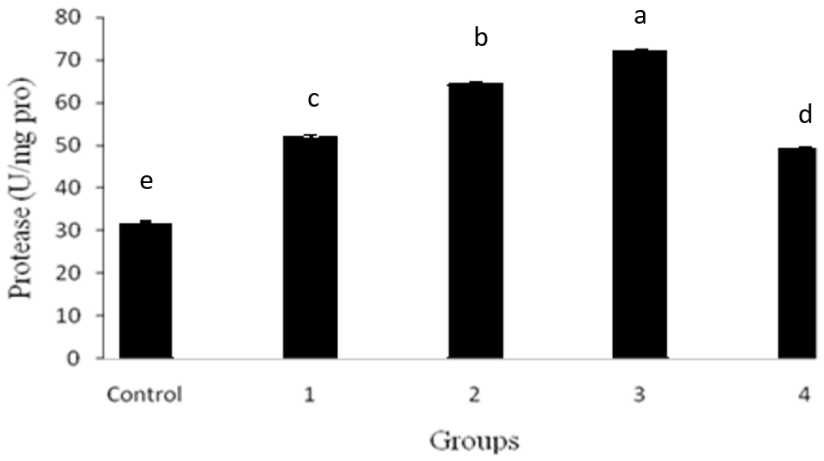

Figure 4. Evaluation of the protease activity of the digestive tract in juvenile rainbow trout (P≤0.5); (n = 18 fish per treatment)

treatment groups (P≤0.05). At the end of the trial, the highest level of growth hormone was found in the 20 KGy group while the lowest was determined in the 40 KGy and control group (P< 0.05) (Figure 5). The difference among the growth hormone values in the 10 KGy and 20 KGy treatment groups was not statistically significant (P> 0.05).

Discussion

Cinnamon is a medicinal plant with health benefits and therapeutic properties. (Arancibia et al., 2014; Ali et al., 2021; Habiba et al., 2021; Velluti et al., 2003). It has shown strong antimicrobial, antiviral, antioxidant and antifungal activities in several studies and

cinnamaldehyde are reported to be the strongest antibacterial component of cinnamon. This plant also contains flavonoids which exhibit antioxidant and free radical scavenging activities (Gruenwald et al., 2010). Cinnamon can increase superoxide dismutase (SOD) activity and decrease malondialdehyde (MDA) levels which are biomarkers for oxidative stress (Tartila et al., 2021). A number of studies have shown mixed results regarding the impacts of irradiation on the quality and chemical composition of different food products and ingredients (Rezanejad et al., 2020; Heidarieh et al., 2021). For instance, γ-irradiation at doses of 10 KGy and 20 KGy reduced the antioxidant capacity of thyme but increased the antioxidant and antibacterial properties of cinnamon (Anwar et al., 2015). Similarly, irradiation at a dose of 10 KGy decreased the total phenolic content and antioxidant activities of rosemary, cumin and black pepper (Hassan et al., 2019), while irradiation of cinnamon at the same dosage increased its phenolic compounds by 6.39%, probably due to the degradation of its tannins (El-megid et al*.,* 2018), and irradiation of it at 25 KGy increased its total flavonoid compounds by 11.19% (El-niley and Farag, 2012). Overall, γ-irradiation appears to enhance the positive qualities of cinnamon.

In the present study, the inclusion of 10 g/kg of irradiated CEE in the fish diet increased final weight and SGR, and decreased FCR in rainbow trout. The growth-promoting effects of irradiated CEE at a dose of 10 KGy were more prominent. The results were consistent with findings from earlier studies on the effects of cinnamon supplementation in poultry and aquaculture (Toghyani et al., 2011; Abdel-Tawwab et al., 2018; Habiba et al., 2021). In a study by Habiba et al., (2021), the effects of dietary supplementation of cinnamon in the fish meal on final weight, SGR, protein efficiency rate and FCR in European sea bass were studied. Results showed that the dietary supplementation of 10 g/kg of cinnamon in fish meals was more affected than the dosages of 0 and 20 g/kg (Habiba et al., 2021). Supplementation of cinnamon in the diet of the rainbow trout (*Oncorhynchus mykiss*) at the levels of 30 g/kg and 50 g/kg significantly enhanced the growth performance (Ravardshiri et al., 2021), while in our study; similar results were obtained using 10 g/kg of irradiated cinnamon in the fish diet. In contrast, the addition of trans-cinnamic acid to the diet of the rainbow trout did not affect the growth performance (Yilmaz, 2019). The striped catfish (*Pangasianodon hypophthalmus*) that received cinnamon extract within their diets demonstrated higher SGR and final weight but the one that received cinnamaldehyde or cinnamon extract exhibited higher protein retention values than the groups that received cinnamon powder or no cinnamon (Tartila et al., 2021). In poultry, the inclusion of 2 g/kg (500 ppm) of dietary cinnamon or cinnamon oil, respectively, could replace antibiotics as a growth promoter, by increasing body weight and decreasing FCR in broiler chicken (Toghyani et al., 2011; Ciftci et al., 2009). Our study demonstrated that the fish that received irradiated CEE at doses of 10 KGy and 20 KGy had higher levels of growth hormone in their blood. This was in agreement with other studies (Habiba et al., 2019). The increased growth performance in the rainbow trout may partially be ascribed to the elevated growth hormone levels.

The chemical composition of fish is important to customers. They prefer to fish with higher protein and omega-3 contents (Ravardshiri et al., 2021). In this study, there were no significant differences in the ash, crude protein and dry matter contents between the control and different experimental groups. The crude protein content was slightly higher in the 10 KGy group, and fat content slightly increased in all groups that received irradiated CEE supplements. The results are in agreement with previous studies on European sea bass (*Dicentrarchus labrax*), common carp (*Cyprinus carpio*), striped catfish (*Plotosus lineatus* (Thunberg, 1791)) and Nile tilapia (*Oreochromis niloticus*) (Habiba et al., 2021; Mohammad et al., 2021; Yilmaz et al., 2019; Tartila et al., 2021; Ahmad et al., 2011), while in the other

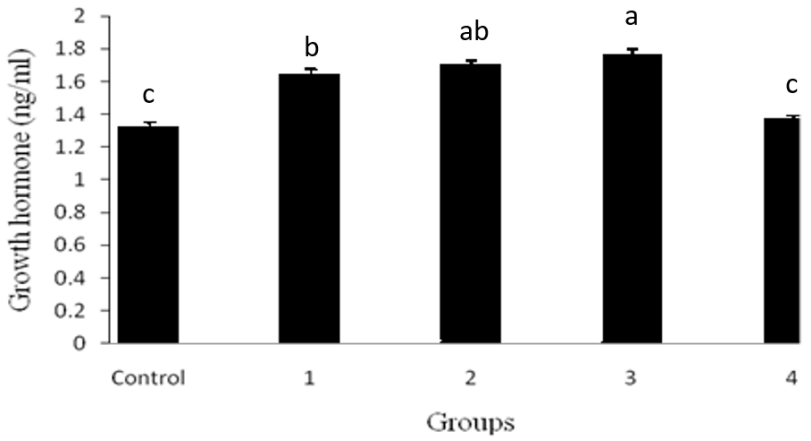

Figure 5. Evaluation of the changes in the serum concentration of growth hormone (GH) in juvenile rainbow trout (P≤0.5); (n = 18 fish per treatment).

research, the ash content increased significantly in the Nile tilapia (*Oreochromis niloticus*) that received dietary cinnamon nanoparticles (Abdel-Tawwab et al., 2018).

In the current experiment, hematocrit increased in all groups that received the CEE, except the group that received irradiated CEE at a dose of 40 KGy. Irradiated CEE did not significantly impact on the RBC, HB or WBC levels. This was in agreement with a previous study where 30 g/kg and 50 g/kg of cinnamon did not improve blood markers in rainbow trout, which is considered normal since cinnamon is not known to affect hematological markers (Ravardshiri et al., 2021). However, these blood parameters improved in Nile tilapia fed with diets containing at a level of 1% cinnamon (Ahmad et al., 2011). In another study, the addition of 1000 and 1500 g/Ton of cinnamon powder in the diet of the rabbits increased RBC, Ht and albumin values (El-kholy et al., 2012). Moreover, the elevation of HB, Hct and RBC and WBC values was reported in the European sea bass (*Dicentrarchus labrax*) treated with 10-15 g/kg of cinnamon (Habiba et al., 2021). The contradictions could be attributed to the differences in species, various environmental factors and preparation methods of cinnamon extract.

Regarding the biochemical markers, there were no significant changes in the levels of total protein, urea and creatinine, indicating the absence of kidney damage (El-megid et al., 2018). The blood glucose levels decreased significantly in the groups that received irradiated CEE at doses of 10 KGy and 20 KGy. This was in agreement with previous studies where cinnamon demonstrated significant glucose-lowering effects in Nile tilapia (*Oreochromis niloticus*) (Ahmed et al., 2021), Rainbow trout (*Oncorhynchus mykiss*) (Ravardshiri et al., 2021) and broiler chickens (Shirzadegan, 2014). The irradiated CEE also decreased serum glucose levels and increased insulin in diabetic rats at a dosage of 10 KGy. The phenolic and polyphenolic compounds of cinnamon are reported to be mainly responsible for blood glucose reduction by mimicking insulin signaling (El-megid et al., 2019). Serum total lipids increased in all treatment groups that received CEE, mainly in the 20 KGy group. This was consistent with a prior study on the effects of cinnamon on European sea bass (Habiba et al., 2021), but in conflict with a previous study that indicated cinnamon to have lipid-lowering effects (Ravardshiri et al., 2021).

In agreement with a previous study (Habiba et al., 2021), no consistent trends were found in data regarding the biochemical markers of liver function (serum AST, ALT and ALP) in the present study. Nonetheless, the AST and ALT still had higher values in the 10 KGy group compared with the other treatment and control groups (110.47 and 28.22, respectively). These enzymes are involved in liver detoxification, and the lack of significant differences between control and treatment groups in this area indicates the stability of health status and the absence of liver failure or biliary system damage in fish (Habiba et al., 2021). Similarly, cinnamon exposure reduced the levels of these enzymes in Nile tilapia (Ahmad et al., 2011) and rainbow trout (Yilmaz et al., 2019). In the present study, Irradiated CEE at the doses of 10 KGy and 20 KGy increased the activity levels of lysozyme, as a marker for innate immunity. Similarly, the lysozyme activity levels were markedly higher in the European sea bass fed cinnamon (15 g/kg), than in lower or higher doses (Habiba et al., 2021). Lysozyme is a bactericidal enzyme that targets peptidoglycan in bacterial cell walls and induces cell lysis (Brott and Clarke, 2019; Chipman et al., 1969). Therefore, increased lysozyme activity indicates that cinnamon extract can enhance cell wall damage in pathogens and strengthen the innate immune responses in fish. In this study, supplementation of 10 g/kg of non-irradiated and irradiated CEE increased the levels of digestive enzymes (amylase, lipase and protease) at doses of 10 KGy and 20 KGy, but their levels decreased again at a dose of 40 KGy of gamma rays. This is consistent with Abdel-Tawab's study where 3-10 g/kg of dietary cinnamon nanoparticles maximized the levels of digestive enzymes (Abdel-Tawwab et al., 2018). Also, digestive enzymes significantly increased in the grass carp (*Ctenopharyngodon idella*) that received dietary cinnamaldehyde supplements, mainly at the level of 108 mg/kg (Zhou et al., 2020). But some papers reported that dietary trans-cinnamic acid had no impact on the activities of the digestive enzymes (Yilmaz et al., 2019). Thus, these results suggest that dietary supplementation of cinnamon can enhance digestion and absorption capacities, leading to improved growth performance.

In conclusion, this study presented the beneficial effects of dietary supplementation of γ-irradiated and non-irradiated cinnamon extract in juvenile rainbow trout. Supplementation of γ-irradiated CEE in the fish diet increased their growth performance, final weight, SGR, digestive enzyme levels and serum lysozyme activity, and decreased their glucose levels, probably due to cinnamon's bioactive compounds. These results suggest that γ-irradiated CEE can be a potential substitute for the antibiotic growth-promoters as it benefits growth performance, enhances the innate immune responses and lacks the adverse effects of other growth promoters, such as antibiotic resistance, higher price/financial costs, toxicity and environmental contamination. The optimal gamma irradiation dosage for 10 g/kg of CEE was 10 KGy, based on our results. Though the use of this gamma irradiated cinnamon extract requires more extensive studies and if the results prove successful and the results are standardized the herb can be used as a replacement for synthetic agents.

Ethical Statement

All applicable international, national, and/ or institutional guidelines for the care and use of fish were followed by the authors. The study was also approved by the Ethics Committee of the Faculty of Veterinary

Medicine, University of Tehran, Iran (code: 7506006-6-10).

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Author Contribution

Marzieh Heidarieh: conceptualization, formal analysis, writing original draft, and follow up publication. Marzieh Heidarieh, Saeideh Naeimi: conceptualization, methodology, and investigation. Ava Resae: preparation of extracts. Tahmineh Heidarieh: Investigation and writing original draft.

Conflict of Interest

The authors declare no competing interests.

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