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R E S E A R C H   P A P E R

Cinnamon Promotes Growth Performance, Digestive Enzyme, Blood Parameters, and Antioxidant Activity of Rainbow Trout (*Oncorhynchus mykiss*) in Low-Carbohydrate Diets

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Abstract

This study was conducted to investigate the effect of cinnamon in high- and low-carbohydrate diets on the physiology of rainbow trout (*Oncorhynchus mykiss*) (16.12±1.33 g). Six experimental diets including control/LCarb (200 g/kg carbohydrate), LCarb-3C (200 g/kg carbohydrate, 30 g/kg cinnamon), LCarb-5C (200g/kg carbohydrate, 50 g/kg cinnamon), HCarb (300 g/kg carbohydrate), HCarb-3C (300 g/kg carbohydrate, 30 g/kg cinnamon), and HCarb-5C (300 g/kg carbohydrate, 50 g/kg cinnamon) were formulated to feed fish for eight weeks. The results showed that fish fed dietary LCarb-3C (72.64 g) and LCarb-5C (73.17 g) had higher weight gain as compared with treatments without cinnamon (P<0.05). Blood performance in LCarb-3C (67.10) was significantly higher than the HCarb-3C group (P<0.05). Fish fed dietary LCarb-3C had the best performance so that cinnamon in this group lowered glucose, total cholesterol, and low-density lipoprotein, improved total protein, and high-density lipoprotein contents. Supplementation of this herb also improved protease and lipase in LCarb-3C and LCarb-5C groups as compared with control. Individuals fed supplemented diets but not HCarb had a higher superoxide dismutase activity when compared with the control group (P<0.05). Generally, cinnamon improved parameters in this study in fish fed a low-carbohydrate diet rather than a high-carbohydrate diet.

Introduction

Providing food for humans is one of the most crucial challenges ahead for humanity. This issue has attracted the attention of nations to enhance the number of aquaculture products in their food basket. Furthermore, the high-quality proteins which come from aquaculture products have made the aquaculture industry the most ongoing section in the food industry, with 8% growth each year (FAO 2020). As the profitability of any aquaculture farm relies on growth performance and feed efficiency, any actions for improving these parameters, such as dietary manipulations, can be a crucial step toward achieving sustainability of aquaculture (Asadi *et al.*, 2020; Asgari *et al.*, 2020; Ghosi Mobaraki *et al.*, 2020). Supplementation diet with herbal medicine is one of these approaches because they are rich in various compounds, including phenols, tannins, alkaloids, terpenoids, and polysaccharides (Van Wyk and Wink 2018). Herbal medicine can not only promote growth performance but also can improve antioxidant capacity,

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flesh quality; and stimulate digestive enzymes, appetite, and the immune system. A long list of herbal medicines such as garlic (*Allium sativum*) (Esmaeili *et al*., 2017a; Esmaeili *et al*., 2017b), barberry (*Berberis vulgaris*) (Ramezanzadeh *et al*., 2020a; Ramezanzadeh *et al*., 2020b), dill (*Anethum graveolens*) (Zeilab Sendijani *et al*., 2020), and spotted lady's thumb (*Polygonum minus*) (Adel *et al*., 2020) and others (Citarasu 2010; Elumalai *et al*., 2020) have been formulated to the rainbow trout (*Oncorhynchus mykiss*) diets to improve the growth.

One of the most popular medicinal herbs is cinnamon (*Cinnamomum verum*), which has been consumed for thousands of years. The main chemical constituents of cinnamon are cinnamaldehyde, cinnamyl alcohol, cinnamic acid, and coumarin (He *et al*., 2005). Research in humans and animals showed this herb has many features such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, hypoglycemic effect, and lipid-lowering effect (Goel and Mishra 2020; Pandey *et al*., 2020; Sierra-Puente *et al*., 2020). Researchers observed these effects in chicken (Saeed *et al*., 2018), pig (Cottrell *et al*., 2020), rat (Alsoodeeri *et al*., 2020), and human (Khan *et al*., 2003;

Pandey *et al*., 2020) but no study in fish. Some authors investigated the effect of cinnamon in the growth and immune response of tilapia (*Oreochromis niloticus*) (Ahmad *et al*., 2011).

According to recently published aquaculture status (FAO 2020), the annual global production of rainbow trout as one of the most farmed salmonids worldwide has been 834 thousand tonnes. Iran produces at least 30% of this amount. In this way, according to the Iran Fishery Organization announcement in 2018, rainbow trout is the highest consumed species in this country.

The response of fish to herbal medicine in various levels of carbohydrates is unknown. This issue is more important for rainbow trout, which is sensitive to high carbohydrate levels in the diet. We hypothesized that cinnamon, as the most popular herb for glucose-lowering and lipid-lowering effect, can alleviate the adverse impacts of including high levels of carbohydrate in diets. In this way, this study was designed to examine the impact of this herb on growth performance, body composition, blood biochemistry, hematology, digestive enzymes, and antioxidant system of rainbow trout.

Table 1. Formulation and proximate analyses of the experimental diets containing different carbohydrates and cinnamon levels.

	Control (LCarb)	LCarb-3C	LCarb-5C	HCarb	HCarb-3C	HCarb-5C
Ingredient			g/kg, as-fed basis			
Fish meal	440	440	440	440	440	440
Soybean meal	200	200	200	180	180	180
Meat and bone meal	70	70	70	70	70	70
Wheat flour	57.3	57.3	57.3	177.3	177.3	177.3
Fish oil	60	60	60	10	10	10
Soybean oil	60	60	60	10	10	10
Lecithin	5	5	5	5	5	5
Dicalcium phosphate	5	5	5	5	5	5
Mineral premix [†]	30	30	30	30	30	30
Vitamin premix [‡]	20	20	20	20	20	20
Antifungus [§]	2.5	2.5	2.5	2.5	2.5	2.5
Antioxidant [¶]	0.2	0.2	0.2	0.2	0.2	0.2
Cinnamon [¶¶]	0	30	50	0	30	50
Filler (starch)	50	20	0	50	20	0
Proximate composition (g/kg dry matter)						
Cinnamaldehyde	0.03	0.49	0.82	0.02	0.53	0.89
Crude protein	404	405	406	403	406	397
Crude lipid	180	184	178	84	79	81
Ash	121	118	119	127	120	124
Carbohydrate [††]	197	196	201	297	301	298
Moisture	98	97	96	89	94	100
Crude fiber	24.9	33.8	39.5	25.2	33.1	41.3
Gross energy (kJ/g) [‡‡]	19.97	20.14	20.24	17.77	17.54	17.60

Diet components were purchased from the Mazandaran Animal & Aquatic Feed (Sari, Mazandaran, Iran).

[†] 1 kg Mineral Supplementation contained: co, 100; I, 400; se, 20; Zn, 10,000; Fe, 6,000; Cu, 600; Mn, 5,000

[§] 5 kg Vitamin Supplementation 0.5% contained: vitamin A 80,000 IU/ kg; vitamin D3 2,000 IU/kg; vitamin k 20 mg/kg; thiamin 60 mg/kg; riboflavin 60 mg/ kg; pyridoxine 100 mg/kg; pantothenic acid 150 mg/kg; niacin 300 mg/kg; biothin 2 mg/kg; folic acid 20 mg/kg; vitamin B12 0.1 mg/kg; inositol 300 mg/kg; ascorbic acid 600 mg/kg; choline chloride 3000 mg/kg.

[§] Anti fungi: Toxiban premix (Component: Alomino silicate, zeolite, bentonate, propionate ammonium).

[¶]Antioxidant: Butylated hydroxytoluene (BHT).

[¶¶] Cinnamon contained 101.0 g/kg moisture, 44.1 g/kg protein, 21.4 g/kg fat, 32.6 g/kg ash, and 282.3 g/kg fiber.

[††] Carbohydrate=100 - (crude protein + crude lipid + ash + moisture).

[‡‡] Estimated gross energy was calculated based on 1g crude protein being 23.6 kj, 1g crude fat being 39.5 kj, and 1g carbohydrate being 17.2 kj. NRC (2011).

Dietary treatments with different levels of carbohydrate and cinnamon: control/LCarb (200 g/kg carbohydrate), LCarb-3C (200 g/kg carbohydrate, 30 g/kg cinnamon), LCarb-5C (200g/kg carbohydrate, 50 g/kg cinnamon), HCarb (300 g/kg carbohydrate), HCarb-3C (300 g/kg carbohydrate, 30 g/kg cinnamon), and HCarb-5C (300 g/kg carbohydrate, 50 g/kg cinnamon).

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Material and Methods

Analyzing Cinnamaldehyde in Diets

As cinnamaldehyde forms 65%-80% of active compounds of cinnamon powder (Rao and Gan 2014), we measured it in diets. Cinnamaldehyde contents in cinnamon powder from the present study was 15.7 mg/g powder. High performance liquid chromatography (HPLC) method was used, and cinnamaldehyde was detected at 275 nm, and peak area was used for signals evaluation (Lungarini *et al.*, 2008). The cinnamaldehyde contents of the experimental diets are reported in Table 1.

Diet Preparation

Six isonitrogenous (400 g crude protein/kg feed) diets were formulated by Lindo software. The diet components were purchased from the Mazandaran Animal & Aquatic Feed (Sari, Mazandaran, Iran). Fresh-powdered cinnamon also was purchased from a local market (cinnamaldehyde contents were measured from this source). The treatments included control (200 g/kg carbohydrate, 0 g/kg cinnamon), LCarb-3C (200 g/kg carbohydrate, 30 g/kg cinnamon), LCarb-5C (200g/kg carbohydrate, 50 g/kg cinnamon), HCarb (300 g/kg carbohydrate, 0 g/kg cinnamon), HCarb-3C (300 g/kg carbohydrate, 30 g/kg cinnamon), HCarb-5C (300 g/kg carbohydrate, 50 g/kg cinnamon). As a previous study showed the maximum growth with the maximum powder dosage (15 g/kg diet) in fish plus a chicken study (Toghyani *et al.*, 2011), we selected upper levels (30 and 50 g/kg diet) to illustrate the optimum dose of cinnamon powder for rainbow trout. The dietary ingredients were first dried and mixed carefully and became homogeneous. After complete mixing, liquid ingredients such as Kilka fish oil, soybean oil, and lecithin were weighed carefully and added gradually to the mixture. The resulting mixture was compressed by a meat grinder (Electrokar EC-1, Tehran, Iran) to form pellets with a 3 mm diameter. Then, pellets were spread out on a tray and dried in an oven to ≥ 90% dry matter at 60°C for 24-48 h. After drying the feeds, packed in suitable packages and kept at 4 °C. The chemical compositions of experimental diets are presented in Table 1.

Fish and Experimental Conditions

For this experiment, a total of 360 juvenile rainbow trout (initial weight: 16.12±1.33 g) were obtained from the Rangin Kaman farm (Sari, Mazandaran, Iran) and adapted two weeks with the experimental condition in Danesh Abzian Arya Mazand (Sari, Mazandaran, Iran). Eighteen 300-L fiberglass tanks within a semi-recirculating system were adjusted for this experiment for six treatments. Throughout the trial, tank water was siphoned off daily 30-40% of water to remove feces and debris. Water quality parameters were regularly checked and kept in the standard range for the culture of rainbow trout throughout the 8-week experiment (temperature 14±0.6°C; DO 7.4±0.35 mg/L; pH 7.20±0.3). Photoperiod was maintained at 12D:12L, and fish were hand-fed three times daily to apparent satiation. The temperature was measured by mercury thermometer (Zomorodazma Company, Iran), dissolved oxygen by Cyberscan Eutech instruments (DO 110, Singapore), and pH by a pH meter (Hanna instrument, 8314, USA) (Hosseinpour Aghaei *et al.*, 2018). Ammonia, nitrite, and nitrate (0.11, 0.01, and 4.9 mg/L) were measured by ASTM International D1426-08 and D3867-09, respectively.

Table 2. Growth performance of rainbow trout fed experimental diets containing carbohydrate and cinnamon for eight weeks.

Growth index	Control (LCarb)	LCarb-3C	LCarb-5C	HCarb	HCarb-3C	HCarb-5C
Initial weight (g)	16.31	16.02	16.21	15.89	16.28	16.04
Weight gain (g)	54.86±2.74 [b]	72.64±1.55 [a]	73.17±5.62 [a]	53.23±5.01 [b]	53.76±2.09 [b]	58.07±2.26 [ab]
SGR (%/day)	2.63±0.24	3.05±0.21	3.04±0.28	2.62±0.18	2.60±0.17	2.73±0.14
FCR	1.07±0.11	0.94±0.12	0.97±0.06	1.05±0.10	1.09±0.09	0.99±0.12
Feed consumption (FC)	58.47±3.21 [b]	68.25±3.47 [a]	71.27±2.50 [a]	55.87±3.98 [b]	57.51±4.11 [b]	56.50±3.55 [b]
DFI (%/day)	2.39±0.53	2.33±0.62	2.41±0.45	2.35±0.36	2.38±0.24	2.24±0.21
Protein efficiency	2.30±0.27	2.63±0.24	2.53±0.25	2.36±0.20	2.26±0.15	2.54±0.16
Lipid efficiency	5.18±0.60	5.78±0.54	5.76±0.71	5.18±0.54	5.13±0.64	5.58±0.57
HSI (%)	1.12±0.09	1.19±0.08	1.01±0.04	1.17±0.06	1.09±0.08	1.15±0.07
VSI (%)	11.54±0.36	11.10±1.06	11.68±1.14	11.52±0.76	11.16±0.65	11.49±0.82
Condition factor	1.04±0.13	1.12±0.05	1.11±0.06	1.10±0.03	1.02±0.05	1.05+0.07
Survival rate (%)	100	100	100	100	100	100

Feed intake (%body weight.day^{-1}) =100× feed consumed (g)/((initial weigh+ final weight) ×0.5×days)

WG (%) = 100 × ((final weight – initial weight) / initial weight)

CF: Condition Factor = (final weight (g) / Length^3) × 100

SGR: Specific Growth Rate = ((Ln final weight – Ln initial weigh) / During the total experimental period (56 days)) × 100

FCR: Feed Conversion Ratio = Dry feed consumed (g) / WG (g)

PE/LE: Protein/Lipid Efficiency Ratio= Weight gain (g) / consumed protein/lipid (g)

HSI: Hepatosomatic Index = (Liver weight (g) / Body weight (g)) × 100

VSI: Viscerosomatic Index = (Visceral weight (g) / Body weight (g)) × 100

Survival (%) = (Number of fish in each group remaining at the end of experiment / initial number of fish) × 100

Values are represented means±SDM of triplicate tanks; means without letter labels are not significantly different. The letters a, and b indicate significant differences in the treatments according to Duncan's multiple range tests (*P*<0.05).

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Growth Performance

At the end of the feeding trial, all fishes were fasted for 24 hours and were then anesthetized with the clove oil stock solution (50-70 ppm) (Esmaeili *et al.*, 2017b). The survival rate and growth indices including feed consumption (FC), weight gain (WG), specific growth rate (SGR), FCR, protein efficiency (PE), lipid efficiency (LE), hepatosomatic index (HSI), visceral index (VSI), and condition factor (CF) were determined using standard methods and relationships. All the applied formulas are reported in the footnote of Table 2. Also, three fishes per tank were randomly selected, and then their respective liver was sampled and weighed. We used whole fish, including its liver, for performing body composition analysis.

Chemical Analysis

The proximate composition of whole-body tissue samples was analyzed using AOAC methods (AOAC 2000). Briefly, crude protein was determined by the Kjeldahl method, using an automatic Kjeldahl system (Kjeltec Analyser unit 2300, Sweden). Crude lipid was analyzed with the Soxhlet extraction method (Soxtec 2050 FOSS Model, Switzerland). Moisture was determined by drying samples in an oven at 105°C for 12 h. A Nabertherm muffle furnace (Model K, Germany) was used for the determination of ash (550°C for 4 h). Fiber content was analyzed with a fiber analyzer (VELP®

Scientifica, Italy). Nitrogen-free extract plus fiber, representing carbohydrate, was calculated using the formula: carbohydrate=100 − (protein + fat + ash + moisture) (Aksnes and Opstvedt 1998). Gross energy of the diet was calculated according to the National Research Council (NRC 2011):

$$\text{Energy (MJ/kg)=(protein×23.6kJ/g)+(fat×39.5kJ/g)+} \\ \text{(carbohydrate×17.2kJ/g) (Figure 1).}$$

Digestive Enzyme Activities

The stomach and whole intestines of each fish were homogenized on ice in an electric homogenizer. Amylase, lipase, and protease measurements were done in the collected digestive tracts from each fish. The total protein content of the supernatant was analyzed using the Bradford method (Bradford 1976), and bovine serum albumin (BSA) was used as a standard. Protease activity was measured as described previously (Hidalgo *et al.*, 1999) using casein hydrolysis at pH 8. Enzyme reaction mixtures consisted of 1% (w/v) casein in water (0.25 ml), buffer (0.25 ml), and enzyme sample (0.1 ml) and were incubated for 1 h at 37 °C. The reaction was stopped by adding 0.6 ml of 8% (w/v) trichloroacetic acid. After holding for 1 h at 2°C, samples were centrifuged at 1800×g for 10 min, and the absorbance of the supernatant was recorded at 280 nm. Tyrosine was used as standard, and one unit of enzyme activity was defined as the amount of enzyme needed to catalyze the

Table 3. Proximate composition of whole-body (g/kg dry matter basis) of rainbow trout (*Oncorhynchus mykiss*) fed containing carbohydrate and cinnamon for eight weeks

	Control (LCarb)	LCarb-3C	LCarb-5C	HCarb	HCarb-3C	HCarb-5C
Protein	657.4±10.6	668.9±17.2	652.2±18.4	659.8±12.7	656.7±13.2	664.1±11.8
Lipid	251.7±22.3 [ab]	239.3±24.5 [b]	253.3±16.4 [ab]	269.7±23.6 [a]	257.7±17.4 [ab]	258.2±15.5 [ab]
Ash	79.6±7.9	77.3±7.2	76.7±8.3	82.5±6.4	76.8±6.7	81.7±7.9
Moisture	734.2±12.1	735.2±18.7	729.8±11.9	720.4±16.1	728.7±11.3	730.5±10.4

Values are represented means±SDM of triplicate samples; means without letter labels are not significantly different. The letters a, and b indicate significant differences in the treatments according to Duncan's multiple range tests (*P*<0.05).

Table 4. Hematological parameters of rainbow trout fed experimental diets containing carbohydrate and cinnamon for eight weeks.

Hematology	Control (LCarb)	LCarb-3C	LCarb-5C	HCarb	HCarb-3C	HCarb-5C
RBC (X10^6/mm^{-3})	1.10±0.13	1.07±0.12	1.06±0.09	0.98±0.13	0.99±0.21	1.11±0.18
WBC (X10^3/mm^{-3})	19.95±1.41	22.20±0.58	22.68±1.04	21.35±1.57	20.34±2.31	19.92±1.89
Haematocrit (%)	30.54±2.76	30.60±2.79	29.47±2.12	29.96±2.40	30.03±3.44	30.23±2.55
Haemoglobin (g/dl)	9.12±0.64	9.98±0.72	9.28±0.79	9.09±1.82	8.96±0.72	9.41± 1.02
MCV (fl)	277.63±29.11	285.98±50.55	278.02±27.84	305.71±36.06	303.33±32.45	272.34±25.77
MCH (pg)	82.91±22.28	93.27±22.72	87.55±20.43	92.44±15.54	90.50±11.56	84.77±12.69
MCHC (%)	29.86±12.54	32.61±9.83	31.49±5.26	30.24±6.05	29.84±8.14	31.13±7.50
Blood performance	62.70±3.40 [ab]	67.10±2.74 [a]	65.73±2.92 [ab]	63.38±2.55 [ab]	62.47±3.11 [b]	62.91±2.00 [ab]

Mean corpuscular volume (MCV) (fl)= (Haematocrit/(RBC X10^6/mm^{-3})) × 10
Mean corpuscular haemoglobin (MCH) (pg)= Haemoglobin/ RBC X10^6/mm^{-3}× 10
MCHC = Haemoglobin/Haematocrit × 100
Blood performance=RBC (X10^6/mm^{-3}) + WBC (X10^3/mm^{-3}) + Haematocrit (%) +Haemoglobin (g/dl) + total protein (g/dL).
Blood performance was introduced by Moha Esmaeili for the first time (Montazeri Parchikolaei et al., 2021).
Values are represented means±SDM of triplicate tanks; means without letter labels are not significantly different. The letters a, and b indicate significant differences in the treatments according to Duncan's multiple range tests (P<0.05).

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formation of 1 µg of tyrosine per 1 min. Lipase activity was assayed by using 0.53 mM pnitrophenyl myristate dissolved in 0.25 mM Tris – HCl, 0.25 mM 2 – methoxy ethanol and 5 mM sodium cholate buffer (pH=9.0). Briefly, 5 µL of enzyme extract in 0.5 ml of the substrate was incubated for 15 min at 30°C. The reaction was stopped by the addition of 0.7 mL of acetone: n-heptane (5: 2), the extract centrifuged for 3 min at 6080 g and 4°C, and absorbance was recorded at 405 nm. In the blank, acetone: n-heptane was added to the substrate before the addition of enzyme extract (Iijima *et al.*, 1998). Specific activity (U) was expressed as:

$$\text{Lipase activity} = (A \text{ (sample (280nm))}) \times \text{value} \times 1000 / (15 \times 16500 \times \text{mg protein})$$

Amylase activity was estimated by using 1% starch dissolved in 100 ml buffer containing 20 mM sodium phosphate and six mM NaCl (pH=6.9). Briefly, 250 µL of enzyme extract was incubated for 3 – 4 min at 25°C. This was followed by the addition of 0.5 ml dinitrosalicylic acid (DNS) and incubation for 5 min at 100°C and the addition 5 ml water. Absorbance was recorded from the change at 540 nm, and the quantity of maltose released was determined from a standard curve prepared from maltose solution. One unit was calculated as the quantity of enzyme that released one µmol of maltose in 1 min (Bernfeld 1955; Worthington 1991). Specific activity (U) was expressed as:

$$\text{Amylase activity} = (\text{Maltose released (µmol)}) / (3 \times \text{mg protein})$$

Table 5. Blood biochemistry in the serum of rainbow trout fed experimental diets containing carbohydrate and cinnamon for eight weeks.

Blood chemistry	Control (LCarb)	LCarb-3C	LCarb-5C	HCarb	HCarb-3C	HCarb-5C
Glucose (mg/dL)	85.37±1.75 [a]	65.40±1.32 [bc]	68.61±5.05 [b]	89.96±2.43 [a]	59.76±3.82 [c]	66.32±5.51 [b]
Cholesterol (mg/dL)	95.42±6.44 [a]	71.25±9.32 [c]	79.76±6.79 [bc]	80.10±7.45 [bc]	74.21±6.35 [c]	89.63±7.29 [ab]
Triglyceride (mg/dL)	215.66±23.55	199.88±16.12	214.70±15.00	210.88±19.27	202.76±18.06	205.62±17.45
Total protein (g/dL)	1.99±0.36 [b]	3.25±0.79 [a]	3.24±0.61 [a]	2.00±0.28 [b]	2.15±0.41 [b]	2.24±0.51 [b]
Albumin (g/dL)	2.01±0.50	2.13±0.47	2.05±0.19	2.57±0.34	2.46±0.46	1.90±0.34
HDL (g/dL)	47.46±2.10 [b]	53.66±2.97 [ab]	50.25±3.50 [b]	52.30±2.88 [ab]	56.71±3.24 [a]	50.02±4.04 [b]
LDL (g/dL)	43.22±3.59 [a]	19.75±4.11 [c]	25.69±4.56 [c]	25.50±3.92 [c]	19.91±3.28 [c]	33.04±3.77 [b]
HDL/LDL	1.05±0.29 [c]	2.72±0.26 [a]	1.96±0.15 [bc]	2.05±0.28 [b]	2.85±0.30 [a]	1.52±0.24 [bc]
Insulin (mIU/ml)	10.25±2.22	9.03±1.45	9.83±2.17	10.04±2.75	8.86±3.20	10.02±2.91

Values were represented means±SDM of triplicate tanks; means without letter labels are not significantly different. The letters a, b, and c indicate significant differences in the treatments according to Duncan's multiple range tests (P<0.05).
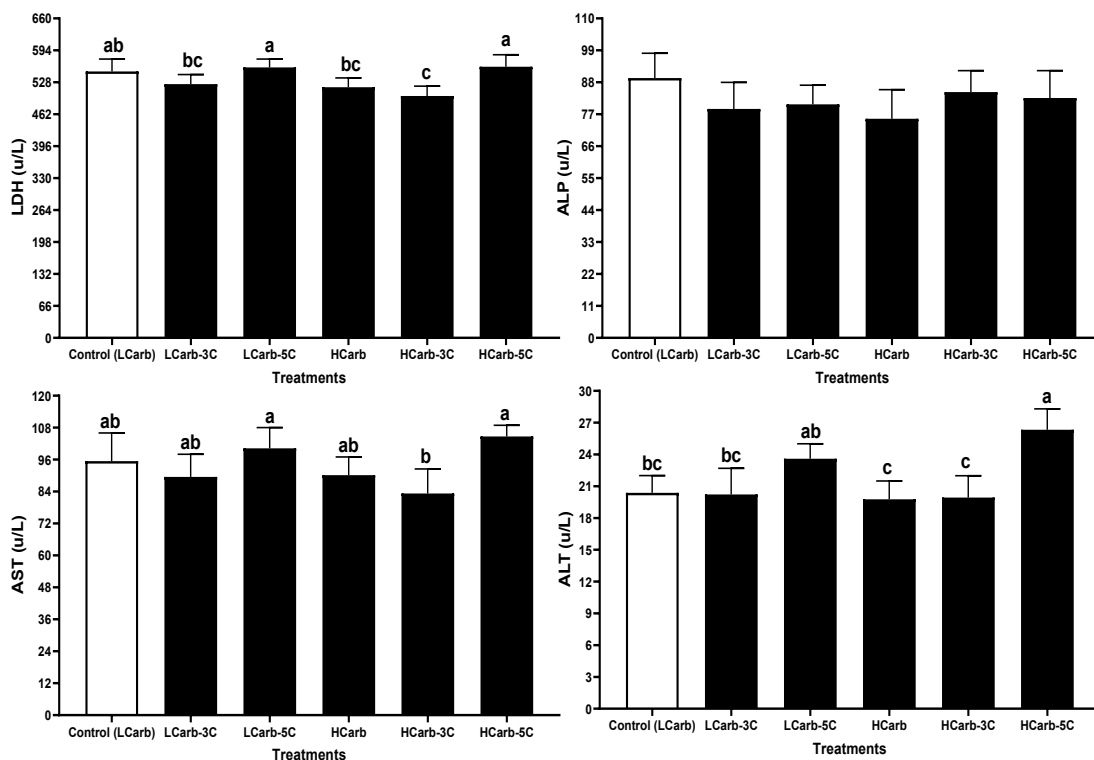

Figure 1. Lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities in serum of juvenile rainbow trout fed experimental diets containing different levels of carbohydrate and cinnamon. Letters a, b, and c indicate significant differences in treatment, according to Duncan's multiple range tests (P<0.05).

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Hematology, Biochemistry, and the Antioxidant Parameters

Blood Collection and Sample Preparation

Seven fish from each tank were sampled for hematological, blood biochemistry, and antioxidant analyses. For preventing stress, fish were anesthetized with a stock solution of clove oil (50 mg/L) (Esmaeili *et al.*, 2017b), and blood samples were collected quickly by venipuncture of the caudal vein using a sterile 5-ml syringe. In the next step, blood was kept in the fridge for 2 h for blood clotting, and then serum was collected after centrifuging in 3000 × g at 4°C (Esmaeili *et al.*, 2017b).

Hematology Profile

Red blood cells (RBCs) were counted in a Neubauer hemocytometer after diluting whole blood with Natt, M.P., and C.A. Herrick solution (1:200), containing 0.1 g of brilliant cresyl blue, 3.8 g of sodium citrate, and 0.2 ml of 37% formaldehyde in 100 ml of distilled water. We counted five central compartments of the middle square of the Neubauer chamber, and the results were multiplied by 10,000. Four marginal squares in the Neubauer chamber were used to count white blood cells (WBCs) after the blood was diluted 1:50 with Natt, M.P., and C.A. Herrick solution, and the results were multiplied by 50 (Kenari *et al.*, 2013). Hemoglobin was measured using the cyanmethemoglobin method. The
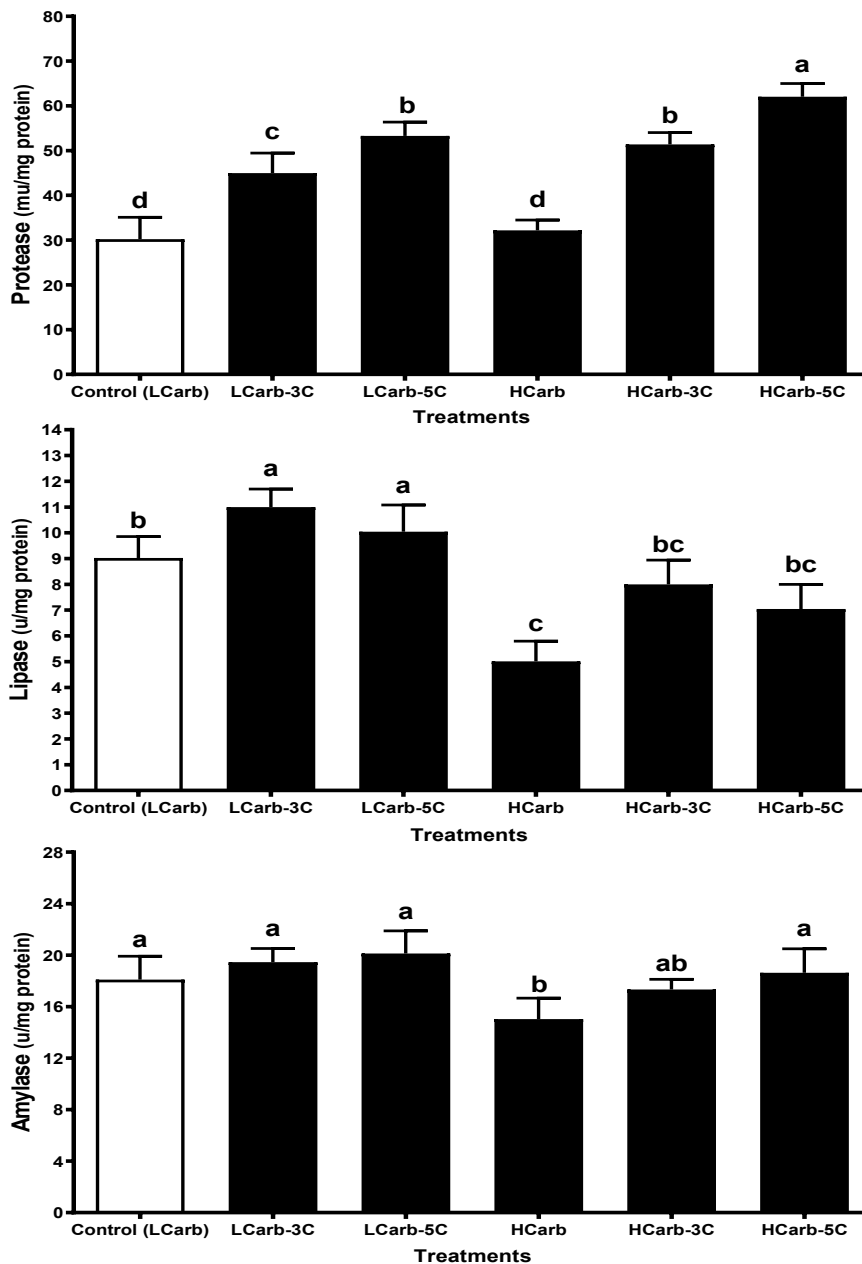

Figure 2. Protease, lipase, and amylase activity of the digestive tract of juvenile rainbow trout fed experimental diets containing different levels of carbohydrate and cinnamon. Letters a, b, c, and d indicate significant differences in treatment, according to Duncan's multiple range tests (P<0.05).

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uncoagulated blood (20 µl) was mixed with 5 ml of Drabkin's solution and placed in a dark place for 5 min. Afterward, it was read (in g/dl) by a spectrophotometer at 540 nm (UNICO, model 2100, USA). Haematocrit (Hct) was determined by the microhematocrit method. First, more than two-thirds of Hct capillary tubes were filled with uncoagulated blood. The tubes were centrifuged at 13000 × g for 5 min in a microhematocrit apparatus, and then hematocrit values were read using a specific graded sheet (Řehulka *et al.*, 2004). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCHC, and blood performance were calculated according to the reported formula in footnote Table 4.

Blood Biochemistry, Liver Enzymes, and Antioxidant Activity

Glucose, cholesterol, triglyceride, total protein, albumin, HDL, and LDL assays were done with the kits from Pars Azmun Company (Pars Azmun, Karaj, Iran) according to the protocols. Insulin was measured with a commercial kit (Fish Insulin, ELISA Kit, CUSABIO) according to the protocol. LDH, ALP, ALT, and AST in serum were measured with an autoanalyzer (Eppendorf, EPOS, Germany). The antioxidant enzymes (SOD and

CAT) in serum were determined using an analysis ELISA kits (ZellBio, GmbH, Germany) according to the protocol.

Statistical Analysis

Our experiment was designed and adjusted according to a completely randomized design. Shapiro–Wilk and Levene's tests were used to test for normality and homogeneity of variance, respectively. All data were analyzed by one-way analysis of variance (ANOVA) using SPSS (version 22.0 for Windows). Duncan's multiple range tests were used to assess differences among six treatments in growth performance factors, body composition, hematology, blood biochemistry, digestive enzymes, and antioxidant activities.

Results and Discussion

Growth Performance

Although numerous studies investigated the effect of cinnamon on animal and human physiology and growth, the exact mechanism is unknown. Cinnamon, as the most well-known herbal medicine for glucose-lowering and lipid-lowering effect, was selected for this
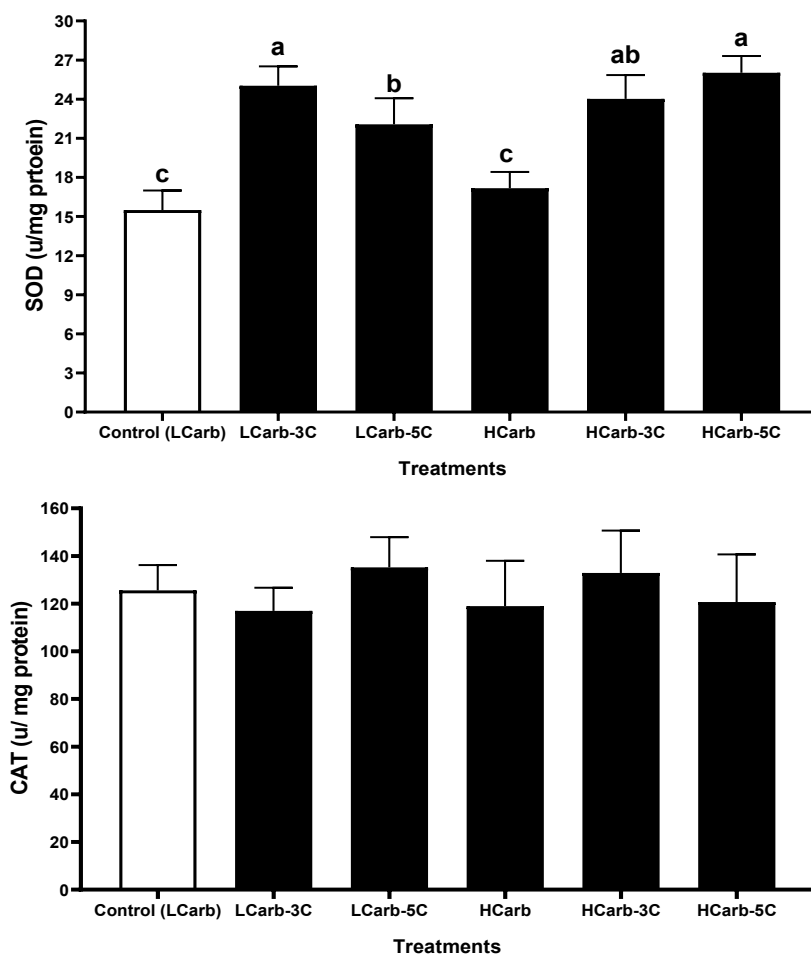

Figure 3. SOD and catalase activity in serum of juvenile rainbow trout fed experimental diets containing different levels of carbohydrate and cinnamon. Letters a, b, and c indicate significant differences in treatment, according to Duncan's multiple range tests (P<0.05).

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research. Also, we selected rainbow trout as they cannot tolerate high carbohydrate levels in the diet. According to Table 2, there was no significant difference in SGR, FCR, DFI, protein and lipid efficiency, HSI, VSI, CF, and survival rate values among treatments. Rainbow trout fed dietary LCarb-3C (72.64) and LCarb-5C (73.17) had significantly higher numbers of WG (g) as compared with others but not HCarb-5C (58.07) ($P<0.05$) (Table 2). Although the difference was not significant, FCR in LCarb-3C (0.94) was lower than the control (1.07) and HCarb-3C (1.09) group (Table 2). Also, FC followed a trend like WG, and it shows fish that ate more feed grew more. In tilapia (10 g/kg nanocinnamon) (Abdel-Tawwab *et al*., 2018; Ahmad *et al*., 2011), chicken (0.25-1 g/kg powder) (Singh *et al*., 2014; Toghyani *et al*., 2011), and quail (200 mg/kg cinnamon oil) (Mehdipour *et al*., 2013) similar outputs were observed. Also, researchers supplemented cinnamaldehyde (the main active component of cinnamon) to fish diets, and as a result, growth, and amino acid transporters genes were overrepresented (Zhou *et al*., 2020). By looking at the literature, a wide range of variety can be seen in optimum dosage showing more research is required.

The main reason for improving growth performance by cinnamon in our study is unknown. Some ideas can be proposed, (i): improved digestion and nutrient digestibility leading to improved nutrient utilization. Digestive enzyme activity somehow confirmed this hypothesis; (ii): balanced lipid and carbohydrate hemostasis. Blood chemistry results confirmed this issue similar to several works in other animals and human (Goel and Mishra 2020; Khan *et al*., 2003; Sierra-Puente *et al*., 2020); (iii): well-known as a potent antioxidant and immune stimulator in fish in other animals (Abdel-Tawwab *et al*., 2018; Ahmad *et al*., 2011; Toghyani *et al*., 2011) which can indirectly improve growth as it happened in our study. However, more digestibility, hormonal, and bacterial studies are needed to illustrate the aforementioned phenomena. The present research is preliminary work, and in future investigations, we would focus on these points. Supplementation of too much cinnamon cause bitterness and astringency taste and potentially reduce feed intake (Bennick 2002). However, in rainbow trout, no sign of adverse effect on feed intake was observed, and it seems that fish can digest 50 g/kg cinnamon without any problem related to palatability. Rainbow trout were fed in apparent satiation levels to make sure we can monitor any adverse effect of cinnamon on feed intake. Interestingly, no study was found in other animals to report decreasing feed intake with supplementing too much cinnamon to the experimental diets. Accordingly, this herb increased feed intake in mice by increasing the neuropeptide Y gene expression in the hypothalamus (Ogawa *et al*., 2020). However, more studies on fish are necessary to illustrate this issue. Growth performance in high-carb diets was not decreased as compared with the control showing that rainbow trout can tolerate carbohydrates in diets up to 300 g/kg. However, it worth highlighting that the fiber contents of diets were lower than 40 g/kg. Cinnamon improved growth performance just in LCarb groups. This study is the first work illustrating that the nutrient contents of diets play a crucial role in the growth-promoting effect of herbs. In general, we suggest 30 g/kg in a low carbohydrate diet as the optimum level for providing maximum growth performance in rainbow trout.

Chemical Composition of the Body

Chemical composition of fish is a major determinant for customers as they prefer to buy and eat fish with fewer lipids but high levels of omega 3 as well as more protein contents. A wide range of internal and external factors such as age, gender, size, water quality, season, and geographical differentiate the chemical composition. Still, the main reason usually comes from the diet (Shearer 1994). Rainbow trout was fed by the dietary LCarb-3C had lower values of lipid (239.3 g/kg) in comparison with HCarb treatment ($P<0.05$) (Table 3). Also, there was no significant difference in protein, ash, and moisture contents among the treatments. Lipid composition is one of the main indicators of the flesh quality of aquatic animals, as they are the most abundant source of omega 3 (Innes and Calder 2020). The result of the present study showed consistency with other data in barberry (Ramezanzadeh *et al*., 2020b) and garlic (Esmaeili *et al*., 2017a). However, some studies reported increased lipid contents in the body when fish fed dietary dill (Zeilab Sendijani *et al*., 2020). Blood biochemistry related to lipid metabolism (Ch, TG, and HDL) confirmed these results, and for the first time, we can report the lipid-lowering and glucose-lowering effect of cinnamon in fish. Lipid-lowering effects of cinnamon in other animals and humans were well reported (Rahman *et al*., 2013; Santos and da Silva 2018) (Maierean *et al*., 2017). Although it was not significant, the inclusion of high carbohydrates to rainbow trout diets increased lipid contents in the body, which is compatible (Brauge *et al*., 1994) and incompatible with other studies (Austreng *et al*., 1977).

Hematology and Blood Biochemistry

Hematology and biochemistry parameters in the blood are relevant factors for monitoring fish status during environmental and nutritional changes (Fazio 2019). These parameters in fish were influenced by multiple factors such as species, size, age, physiological status, environmental conditions, and diet (quantity and quality, basal diet ingredients, protein source, vitamins, herbal medicine). The cinnamon inclusion did not elevate the RBC, WBC, hematocrit, hemoglobin, MCV, MCH, and MCHC contents. However, blood performance in rainbow trout fed dietary LCarb-3C (67.10) was significantly higher than those that were fed by the HCarb-3C diet ($P<0.05$) (Table 4). Moha Esmaeili

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introduced blood performance as a new hematological factor for aquaculture studies, which is a sum of the RBC, WBC, hematocrit, hemoglobin, and total protein (Montazeri Parchikolaei *et al*. 2021). Interestingly, this factor worked well in our research as well, so that while there was no significant difference among each element, this factor was significantly higher in the HCarb-3C group (this group had the highest growth as well). In beluga (*Huso huso*) also similar results were observed, and blood performance positively correlated with growth (Montazeri Parchikolaei *et al*., 2021). The results of hematology (no change in RBC, WBC, hematocrit, and hemoglobin) in rainbow trout were not surprising as cinnamon usually is not well-known for improving immunohematology parameters. Like our results, the authors did not find any significant difference in hematological factors in cinnamon-fed chickens (Toghyani *et al*., 2011). However, the LCarb-3C group can be considered as the high-performance treatment regarding hematological factors.

In the present study, cinnamon decreased glucose, cholesterol, and LDL; and increased total protein, HDL, and HDL/LDL ratio. More precisely, individuals fed control and HCarb diets had significantly higher glucose (85.37, 89.96 mg/dL) as compared with those fed cinnamon-supplemented diets, respectively (P<0.05) (Table 5). Also, the control group had the highest cholesterol (95.42 mg/dL) and LDL (43.22) contents as compared to other treatments (P<0.05). Interestingly, the total protein in LCarb-3C (3.25) and LCarb-5C (3.24) groups, which have higher growth as well, was higher than other groups (P<0.05). Finally, cinnamon increased HDL/LDL level in fish fed dietary LCarb-3C (2.72) and HCarb-3C (2.85) as compared to control (P<0.05). Cinnamon is the most well-known herbal medicine for lowering glucose and cholesterol. Several studies in humans and different animals have reported these effects, which we already mentioned to a few of them in the introduction section. Accordingly, similar results were observed in rainbow trout, and for the first time, these effects on fish are reported. Cinnamon improves lipid-related blood biochemistry by directly affecting lipid metabolism. Inhibiting hepatic HMG-CoA reductase activity, which declined cholesterol production in the liver and suppressed lipid peroxidation (Baker *et al*., 2008), has been one of the primary mechanisms. Besides, elevated HDL in our study can be due to increasing lecithin cholesterol acyltransferase activity (crucial for blood lipid regulation) (Nakhjavani *et al*., 2020). This plant mimics the insulin effect via decreasing glucose absorption from the intestine and increasing the expression of insulin receptor genes, IGF1 signaling, and glucose uptake by adipose tissues. However, this herb, with changing more than 30 different pathways, can do the lowering effect of cholesterol and glucose, which was reviewed elsewhere (Mollazadeh and Hosseinzadeh 2016). While needed more research in molecular and gene levels, it seems that in cinnamon-fed fishes, improved blood biochemistry probably has been a

reason for growth improvement through some of the pathways, as mentioned earlier.

Although no trend in data related to liver function enzymes (LDH, ALP, ALT, and AST) in serum was found, rainbow trout fed dietary HCarb-5C and LCarb-5C had higher value of LDH (558.79, 560.20), ALT (23.60, 26.32), and AST (100.25, 104.67), respectively when compared with other groups (P<0.05). These enzymes are involved in the detoxification of the liver, and we can propose that individuals fed the highest level of cinnamon in our study tried to detoxify the liver from some toxic compounds. The toxicity of several toxic compounds in cinnamon was reviewed elsewhere (Higaki *et al*., 2018; Lee *et al*., 2008). However, the contents of toxic compounds perhaps were not too much as the growth in cinnamon-fed fishes was higher. Consequently, LCarb-3C and LCarb-5C treatments were high performance in most of the investigated blood biochemistry factors.

Digestive Enzymes

It is reported that some herbal medicines are able to improve nutrient digestion and absorption. They do it via two main mechanisms, (i): elevation of bile acid secretion that has a key role in lipid digestion and absorption, (ii): facilitating digestion and absorption of proteins, carbohydrates, and lipids by stimulating the activities of the digestive enzymes (Platel and Srinivasan 2004). Generally, cinnamon stimulated the digestive enzyme in rainbow trout. Protease activity (U/mg protein) in the LCarb-3C (44.98), LCarb-5C (53.29), HCarb-3C (51.36), and HCarb-5C (62.03) were statistically higher than the unsupplemented groups (P<0.05) (Figure 2). Fish fed the LCarb-3C (11.00) and LCarb-5C (10.04) diets displayed higher lipase activity than those fed other feeds (P<0.05). Surprisingly, fish fed dietary HCarb (15.03) exhibited a lower content of amylase when compared with other groups but not HCarb-3C (Figure 2). Various factors can affect digestive enzymes, such as light (Cuvier-Péres *et al*., 2001; Hou *et al*., 2019), stocking density (Wang *et al*., 2019), and carbohydrate quantity and quality in diets (Zhang *et al*., 2019) in fish. As temperature, light, and stocking density were similar between treatments, diets were considerably influenced digestive enzymes in the current work. Recently, Zhou *et al*. (2020) showed that cinnamaldehyde increased the digestion and absorption capacity by increasing the activities of intestinal and hepatopancreas digestive enzymes and intestinal brush border enzymes in grass carp (*Ctenopharyngodon idella*). Also, cinnamaldehyde upregulated amino acid transporters (AATs) in the intestine of this fish. Similarly, researchers demonstrated that dietary cinnamon nanoparticles elevated the activities of protease, amylase, and lipase in tilapia (Abdel-Tawwab *et al*., 2018). The results of our study are somehow consistent with their works. One reason for the improvement in digestive enzyme activity in fish fed the cinnamon diets could be attributed to the antioxidant properties of this

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herb. Results of antioxidant activities in the present research are a further line of evidence. Modifying the structure and function of digestive organs by improving antioxidant defense has been well reported (Sen and Chakraborty 2011).

In the present study, however, fish with higher growth performance had higher activity of digestive enzymes as well. Similarly, many investigators reported stimulating digestive enzymes enhanced growth (Esmaeili *et al*., 2017a; Sankar *et al*., 2017), while others found no direct relationship (Lemieux *et al*., 1999; Ramezanzadeh *et al*., 2020b).

Antioxidant Activities

Measuring antioxidant enzymes like SOD and CAT in fish can be useful markers for monitoring fish health status. These days, they are common-investigated factors in aquaculture (More than 2000 records, according to Scopus). SOD and CAT enzymes play vital roles in protecting cells against uncontrolled oxidative processes, leading to radical damage of superoxide and H_2O_2 (Martínez-Álvarez *et al*., 2005). In the present study, SOD activities were significantly higher in fish was fed by cinnamon diets. Precisely, rainbow trout fed dietary control (15.50) and the HCarb (17.16) had substantially lower values as compared with those fed cinnamon diets (P<0.05) (Figure 3). These results also showed increasing carbohydrate levels in diets did not differ SOD and CAT activity (Figure 3). Similar to our works, increasing SOD and/or CAT in rainbow trout fed lemon balm (*Melissa officinalis*) (Bilen *et al*., 2020), lemon verbena (*Aloysia citrodora*) (Hoseinifar *et al*., 2020), rosemary (Karataş *et al*., 2020), and dill (Zeilab Sendijani *et al*., 2020) were reported. Also, Abdel-Tawwab *et al*. (2018) reported an increase in these enzymes when tilapia fed nanocinnamon. On the other hand, so few studies reported decreasing SOD and CAT activities in rainbow trout when they were fed by ginger (*Zingiber officinale*) (Zargar *et al*., 2020). Cinnamon has a strong antioxidant activity, and this effect was reported well in different animals and humans (Abo Ghanima *et al*., 2020; Bastos *et al*., 2017). When we match the antioxidant enzymes and liver function enzymes, it can be concluded that cinnamon helped rainbow trout to keep homeostasis related to oxidative stress at an optimum level. This contributed to fish growing better as in our study and others were observed; higher antioxidants activities were usually in parallel with high growth performance. Also, elevated digestive enzymes were other evidence that oxidative stress was well-controlled with cinnamon and probably caused higher growth performance.

Conclusion

Conclusively, regarding growth performance, body composition, hematological parameters, blood biochemistry, digestive enzymes, and antioxidant

activity, the LCarb-3C group had higher performance. For the first time, we reported the glucose-lowering and lipid-lowering effects of cinnamon in fish. Having a higher level of blood performance, HDL, total protein, digestive enzymes, and antioxidant activity can be some reasons for increasing growth in this treatment. Also, the glucose-lowering and lipid-lowering effects of this herb probably caused rainbow trout to keep optimum metabolic hemostasis for growth. In future research, focusing on digestibility, immune response, and gene expression is suggested to see how these factors contribute to improving growth performance.

Author Contribution

MR: student, lab works, research, drafted version, SB: Main supervisor, design, review, SRJ: drafted version, validation, statistical analysis, MB: drafted version, validation, statistical analysis

Conflict of Interest

There is no conflict of interest to report.

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