Iranian Journal of Fisheries Sciences 22(5) 986-997 2023
DOI: 10.22092/ijfs.2023.130376

Research Article

Effects of different cinnamon powders levels in low and high carbohydrate diets on growth performance and the liver enzymes activity in rainbow trout (*Oncorhynchus mykiss*) juveniles

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Received: January 2020 Accepted: April 2021

Abstract

This study was carried out to evaluate the effect of different cinnamon powder levels in low and high carbohydrate diets on growth performance, liver histological changes, and the liver enzyme activity of rainbow trout (*Oncorhynchus mykiss*) juveniles. For this purpose, 360 rainbow trout with an average initial weight 16.12±1.33 (means±SD) were fed six diets supplemented with Diet1 (200 g/kg carbohydrate), Diet2 (200 g/kg carbohydrate, 30 g/kg cinnamon), Diet3 (200 g/kg carbohydrate, 50 g/kg cinnamon), Diet4 (300 g/kg carbohydrate), Diet5 (300 g/kg carbohydrate, 30 g/kg cinnamon), and Diet6 (300 g/kg carbohydrate, 50 g/kg cinnamon) for 8 weeks The results showed that fish fed Diet2 (62.64) and Diet3 (63.17) had higher weight gain as compared with treatments without cinnamon ($p<0.05$). Alanine aminotransferase and alkaline phosphatase were influenced by the experimental diet and the highest values were observed in Diet3 and Diet6 treatments ($p<0.05$), but the lowest content of aspartate aminotransferase was observed in Diet3 and Diet6 treatments ($p<0.05$). It is concluded that dietary cinnamon can improve growth performance and liver enzyme activity in fish fed a low-carbohydrate diet rather than a high one.

Keywords: Rainbow Trout, Cinnamon, Liver histological, Practical diets

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Introduction

In developed and developing countries, the aquaculture industry plays an important role in providing food, economic growth, and improving the living standards of the people (Desai *et al*., 2012). Rainbow trout (*Oncorhynchus mykiss*) is one of the best commercial fish in the aquaculture industry, which has a valuable share in the human food supply (Talebi *et al*., 2013). Rainbow trout is a carnivorous fish and it needs high dietary protein content to obtain optimal growth. The most expensive component of the fish diet is protein, which increases feed cost. Therefore, the usage of non-protein energy sources in the diet is essential for optimal protein utilization (Wilson, 1994). Numerous studies have shown that protein utilization for growth can improve by replacing dietary protein with non-protein sources such as carbohydrates (Webster and Lim 2002; Mohanta *et al*., 2007). Carbohydrates and lipids are cheaper than protein (Mohammadzadeh *et al*., 2017). It has also been reported that fish have a limited ability to digest carbohydrates and excessive intake of this nutrient may restrict the growth and health of fish (Hemre *et al*., 2002; Zhou *et al*., 2013). In general, rainbow trout as a carnivorous fish has less ability to digest and absorb carbohydrates and they cannot tolerate a high level of carbohydrate in the diet (Hemre *et al*., 2002).

Over the past two decades, the number of studies has increased with the common finding that medicinal plants have the potential to promote growth and increase digistablity in commercial species. One of the most popular medicinal plant is cinnamon (*Cinnamomum verum*) and cinnamaldehyde, cinnamyl alcohol, cinnamic acid, and coumarin are main chemical constituents of this plant (He *et al*., 2005). This herb has many features such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, and lipid-lowering (Pandey *et al*., 2020). However, cinnamon is popular for a hypoglycemic effect and lipid-lowering effect (Goel and Mishra, 2020; Pandey *et al*., 2020; Sierra-Puente *et al*., 2020). These effects were observed in chicken (Saeed *et al*., 2018), pig (Cottrell *et al*., 2020), rat (Alsoodeeri *et al*., 2020), cattle (Sadri *et al*., 2017) and human (Khan *et al*., 2003; Pandey *et al*., 2020). However, some authors investigated the effect of cinnamon in the growth and immune response of tilapia (*Oreochromis niloticus*) (Ahmad *et al*., 2011). Different studies showed that cinnamon reduced fish muscle carbohydrates due to the increased metabolism. Cinnamon improves lipid-related blood chemistry by directly affecting lipid metabolism. Inhibiting hepatic β-Hydroxy β-methylglutaryl-CoA reductase activity, which declined cholesterol production in the liver and suppressed lipid peroxidation has been one of the primary mechanisms (Baker *et al*., 2008). Therefore, it is possible to increase carbohydrate levels in the fish diet by using cinnamon supplementation. Here it was tried to design moderate diets in terms of carbohydrates level for fish to see how cinnamon as the most famous herbal for balancing glucose and lipid hemostasis responds to these diets. So that, the present study was conducted to determine the effect of dietary cinnamon in low and high carbohydrate diets on growth performance and liver enzyme activity of rainbow trout.

Material and method

Experimental diets

Six isonitrogenous (400 g crude protein/kg feed) experimental diets were formulated to contain graded levels of cinnamon in low and high carbohydrate levels. The experimental diets were prepared by mixing the dry ingredients in a mixer. After complete mixing, liquid ingredients such as Kilka fish oil, soybean oil, and lecithin (Table 1) were weighed and added gradually to the dry mixture to form a soft dough. The mixed dough was extruded through an electric meat grinder (Electrokar EC-1, Tehran, Iran) to form pellets with a 3 mm diameter. Then, the pellet was dried at 60°C for 6 h in a drying oven. After drying the pellets, packed in suitable packages, and kept at 4°C. Dietary treatments with different levels of carbohydrate and cinnamon: Diet1 (200 g/kg carbohydrate), Diet2 (200 g/kg carbohydrate, 30 g/kg cinnamon), Diet3 (200g/kg carbohydrate, 50 g/kg cinnamon), Diet4 (300 g/kg carbohydrate), Diet5 (300 g/kg carbohydrate, 30 g/kg cinnamon), and Diet6 (300 g/kg carbohydrate, 50 g/kg cinnamon).

Animals and experimental condition

For this study, 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 transferred to a wet labratory located in Danesh Abzian Arya Mazand (Sari, Mazandaran, Iran). Fish were adapted two weeks with the experimental condition in Danesh Abzian Arya Mazand and then they were randomly divided into eighteen fiberglass tanks (300 L) with 20 fish per tank. After the acclimation period, the tanks were randomly appointed to 6 treatments with 3 replications. During the feeding period, fish were hand-fed with experimental diets three times a day until apparent satiation for 56 days. The total diet offered and weight of feed given each day was recorded for calculating feed conversion rate.

During the experimental period, each fiberglass tank was aerated by using air stones. Each fiberglass tank was cleaned daily, and 30-40% of water volume was renewed by freshwater every day before the first feeding time in the morning. Water quality parameters were checked periodically, pH was found 7.2±0.3, dissolved oxygen was 7.4±0.35 $mg.L^{-1}$, and water temperature was 14±0.6°C.

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

Ingredient	Control (LCarb)	LCarb-3C	LCarb-5C	HCarb	HCarb-3C	HCarb-5C
	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)						
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
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).

†† 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).

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*., 2017). The growth performance and survival rate were calculated as below (Mohammadzadeh *et al*., 2017):

Body weight increase (BWI, %) = 100 × [final weight (g) – (initial weight (g)] / initial weight (g)

Survival rate (%) = 100 × (no. of fish stocked – no. of fish died) / no. of fish stocked

K: Condition Factor = (W2 (g) / Length^3) × 100

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

Hepatosomatic index (HSI) = weight of liver (g) / final weight (g) × 100

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

Liver enzymes

At the end of the feeding trial, all fishes were fasted for 24 hours and 6 fish from each tank were randomly sampled then were anesthetized the clove oil stock (Kralicin) solution (50-70 ppm) (Esmaeili *et al*., 2017). The liver was removed and stored at -80°C for future analysis. Then tissues were weighed maximum up to 0.1 g and homogenized in 2 mL of 0.5 M (pH 7.4) Tris-HCl buffer and finally centrifuged at 8,000 rpm for 25 minutes at 0°C and the supernatant was taken in Teflon tubes for the analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (Samanta *et al*., 2014).

Histological procedures

3 fish from each tank were anesthetized in the clove oil stock (Kralicin) solution (50-70 ppm). Pieces liver was excised, washed in physiological saline, and fixed in aqueous Bouin ́s fluid for 8 hours. The tissues were dehydrated in an ethyl alcohol series of ascending concentrations, embedded in paraffin, and sectioned at 5 mm. The tissue sections were stained with Haematoxiline and Eosin , 10 sections of each fish were examined by light microscope (×100) and (×400). After this analysis, a mean value for the glycogen content for each site was calculated for each treatment (Bozorgnia *et al*., 2016; Hoseinifard *et al*., 2018; Taleghani *et al*., 2019).

Statistical analysis

SPSS software (version 22.0 for Windows) was used to analyze data. Shapiro-Wilk and Levene's tests were applied to check the data normality and homogeneity of variances, respectively. The effect of the treatments on growth performance and metabolic enzymes was examined by two-way ANOVA. Duncan's multiple range tests were used to assess differences among six treatments in growth performance factors and metabolic enzymes (Ahmad *et al*., 2011).

Results

Growth Performance

The growth performance of rainbow trout juveniles fed by experimental diets is presented in Table 2. The survival rate was generally high (100%) over 8 weeks trial and it was not affected by experimental diets (p>0.05). After 8 weeks, there was a significant difference in final weight and body weight increase between treatments (p<0.05). The highest final weight was observed in fish fed with Diet3 treatment. HSI, FCR, SGR, and condition factor were not affected by various dietary carbohydrate and cinnamon levels (p>0.05).

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

Growth index	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6
Initial weight (g)	16.31 ± 0.8	16.02 ± 1.02	16.21 ± 0.75	15.89 + 0.2	16.28 ± 1	16.04 + 0.74
Final weight (g)	54.86 ± 2.74 ^{b}	62.64 ± 1.55 ^{a}	63.17 ± 5.62 ^{a}	53.23 ± 5.01 ^{b}	53.76 ± 2.09 ^{b}	58.07± 2.26 ^{ab}
BWI (%)	168.13 ± 2.86^{b}	195.51 ± 18.32^{a}	194.79 ± 20.52^{a}	167.50 ± 3.91^{b}	165.11 ± 6.62^{b}	180.96 ± 3.97^{ab}
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
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
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
FCR	1.07 ± 0.11	0.93 ± 0.12	0.97 ±0.06	1.05 ± 0.10	1.09 ± 0.09	0.99± 0.12
Survival rate (%)	100	100	100	100	100	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).

Liver enzymes

Effect of various levels of experimental diet on liver enzyme activity of rainbow trout is presented in Table 3. The alanine aminotransferase and alkaline phosphatase were influenced by experimental diets and the highest value was observed Diet3 and Diet6 treatments (p<0.05). The aspartate aminotransferase content was affected by different levels of carbohydrate and cinnamon and the highest value was observed Diet4 (p<0.05).

Table 3: Liver enzyme activity of rainbow trout fed experimental diets containing carbohydrate and cinnamon for eight weeks.

Enzyme activity	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6
ALP	35.73 ± 3.40^{c}	37.25 ± 5.26^{b}	42.67 ± 4.60^{a}	39.60 ±3.46^{ab}	38.21 ± 4.33^{b}	41.54 ±3.43^{a}
ALT	82.38 ± 0.70^{bc}	83.03 ±0.26^{bc}	92.21 ±3.53^{ab}	80.64 ± 0.70^{c}	79.53 ± 4.75^{c}	95.60 ±0.24^{a}
AST	551.34 ±56.196^{a}	404.36 ±19.09^{b}	314.21 ±64.34^{b}	632.08 ±14.14^{a}	532.50 ±38.79^{a}	530.33±12.02^{a}

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).

Histopathology of liver

The main alterations found in the liver were: vacuole degeneration (green arrow), bile duct hyperplasia (blue arrow) (Fig. 1, T4). These alternations were found in Diet4 (T4) treatment (Fig. 1, T4) and they are very week and no serious tissue damage was observed. There is no alteration in other treatments (Fig. 1).

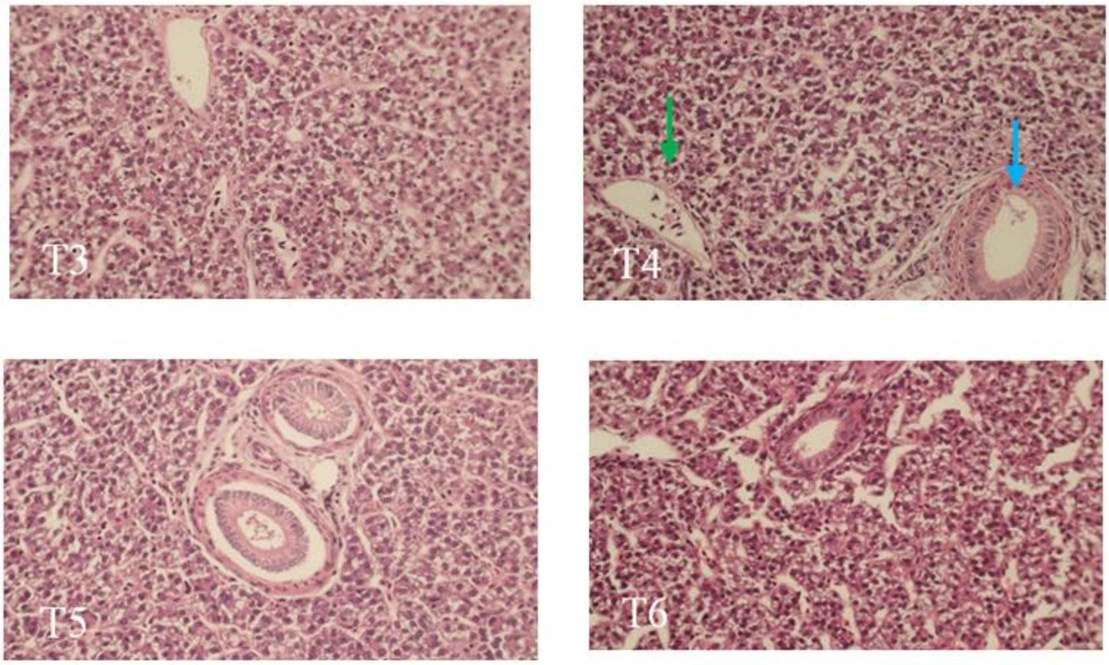

Figure 1: Liver section of Rainbow trout fed different levels of cinnamon in high and low carbohydrate diets. Hematoxylin-Eosin staining (H&E). (× 100) and (× 400). Vacuole degeneration (green arrow), bile duct hyperplasia (blue arrow). T1: Diet1 (200 g/kg carbohydrate), T2: Diet2 (200 g/kg carbohydrate, 30 g/kg cinnamon), T3: Diet3 (200g/kg carbohydrate, 50 g/kg cinnamon), T4: Diet4 (300 g/kg carbohydrate), T5: Diet5 (300 g/kg carbohydrate, 30 g/kg cinnamon), and T6: Diet6 (300 g/kg carbohydrate, 50 g/kg cinnamon).

Discussion

The results of the present study showed that the final weight was improved when the fish were fed with a diet containing cinnamon levels. Rainbow trout fed dietary Diet2 (62.64) and Diet3 (63.17) had significantly higher final weight as compared with others but not Diet6 although different levels of cinnamon led to an improvement in the growth performance on both carbohydrate levels (200 and 300 gr). The Cinnamon leaves contain biologically active compounds including tannins, saponins, flavonoids, and cinnamaldehyde that can act as anti–hypercholesterolemia, and antioxidants (Gruenwald *et al*., 2010). Although numerous studies investigated to improve the growth performance and food conversation of the fish by using cinnamon leaves flour. Setiawati *et al*. (2016) represented that a diet containing cinnamon leaves improved protein retention and feed efficiency. Besides, diets containing 0.1% and 1% cinnamon leaf extract increased specific growth rate, feed efficiency, and protein retention in Asian catfish, *Pangasianodon hypophthalmus* (Setiawati *et al*., 2016). Brauge *et al*. (1994) and Gumus and Ikiz, (2009) suggested that 300 gr of carbohydrate is the upper limit for rainbow trout diets. The findings of the present study are in agreement with the suggestions of Wilson (1994), Medale *et al*. (1994), and Gumus and Ikiz (2009) indicating that the optimal dietary level of digestible carbohydrates for rainbow trout is lower than 30%. Although fish-fed Diet6 containing 300 g/kg carbohydrate, 50 g/kg cinnamon showed higher growth than fish-fed Diet4 containing 300 gr/kg carbohydrate and 0 cinnamon suggesting, the presence of cinnamon in the diet may increase the digestibility of carbohydrates for trout. In tilapia (10 g/kg nanocinnamon) (Abdel-Tawwab *et al*., 2018), 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 results were observed.

It is well established that liver size is directly related to hepatic glycogen level in salmonids (Gumus and Ikiz, 2009). Carbohydrates in the diet as a source of energy can be deposited in the liver as lipids and glycogen after absorption. The HSI value was not changed with the diets containing cinnamon in low and high carbohydrate diet. However, HSI was slightly lower for fish fed with high cinnamon level diets. Cinnamon acts as insulin mimesis that is to increase cellular glucose metabolism so glycogen cannot be deposited in the liver (Broadhurest *et al*., 2000).

In the present study, liver ALP and ALT levels were influenced by the experimental diets and cinnamon could increase these enzymes. Individuals fed Diet3 and Diet6 had a higher value of ALP and ALT when compared with other treatments. These enzymes are involved in detoxification of the liver, and we can propose that individuals fed the highest level of cinnamon in the present 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. Cinnamon could decrease AST levels. Rainbow trout were fed with Diet1 and Diet4 diets had a higher value of AST when compared with other treatments, suggesting an improvement in overall fish health when fish are fed cinnamon-supplemented diets.

Regarding growth performance and liver enzyme activity, the Diet3 containing 200 gr/kg carbohydrate and 50 gr/kg cinnamon had better performance. These results showed that cinnamon could be caused better carbohydrate efficiency in the rainbow trout diet.

Acknowledgment

The authors express gratefulness to the staff at the Rangin Kaman farm Sari, Mazandaran, Iran, for supplying fish and providing necessary facilities for this experiment.

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