Effect of dietary dehydrated sour lemon peel (*Citrus limon*) powder on metabolic enzymes, serum biochemistry and stress status of rainbow trout (*Oncorhynchus mykiss*) juvenile

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Abstract

Lemon peel is by-products contain bioactive compounds that has beneficial effects on fish health. A 45-day feeding trial was conducted to evaluate the potential of dietary dehydrated lemon peel (*Citrus limon*) powder (DLPP) on metabolic enzyme, serum components and stress status of rainbow trout juvenile (initial weight of 26.8±1.9 g). Fish were randomly stocked to four treatments feeding diets containing 0 (control), 0.5, 1.5, and 2.5% of DLPP diets in triplicates. At the end of feeding experiment, metabolic enzyme (Alkaline phosphatase; ALP, Aalanine transaminase; ALT, Aspartate aminotransferase; AST), serum biochemistry (cholesterol, triglyceride, protein and albumin) and stress (glucose and cortisol) were assessed. There was no significant difference in fish growth performance between treatments (p>0.05). Fish fed with 1.5% of DLPP showed a significant decrease in cholesterol and triglyceride (p<0.05). Moreover, serum protein and albumin were improved in fish fed with 1.5% and 0.5% DLPPP, respectively (p<0.05). The highest of AST, ALP and ALT were observed in control group (p<0.05). There were no significant differences in cortisol and glucose of fish between the treatments (p>0.05). Finally, it seems that the inclusion of 0.5 and 1.5% DLPP in diet can improve metabolic enzymes, biochemical parameters and reduced stress of rainbow trout juvenile.

Keywords: Lemon peel, Hepatic enzyme, Biochemical parameters, Stress, Rainbow trout

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important farmed fish worldwide and such Iran, and its production has increased due to of its well-adapted for intensive farming and high commercial value. With the rapid growth of this species in aquaculture industry, the risk of disease outbreaks and mortality rate has increased, and consequently cause enormous economic losses (Stentiford *et al*., 2017). Therefore, the main focus of the aquaculture sector has been on disease prevention. Besides management practices, improving innate immunity is an efficient way to prevent pathogen localization in the fish body (Taheri Mirghaed *et al*., 2018). Farmers often use prophylactic and therapeutic agents on a large scale such as antibiotics in aquaculture. However, antibiotic administration has been limited due to environmental concerns, the rise of resistant strains, and negative impacts on the host (Yousefi *et al*., 2023). Hence, it is important to find secure way to enhancement fish health in aquaculture without side effects and undoubtedly, diet manipulation can be a viable option to resolve this issue.

Studies have shown that phenolic compounds in citrus peel may exert antimicrobial and antioxidant effects when fed to animals (Akbarian *et al*., 2011). Lemon (*Citrus limon*) peel is common by-products of the food and juice extraction industry. During citrus juice processing, a considerable quantity of wastes or by-products is generated. These by-products are available at low cost in most seasons in some countries like Iran. Waste contains 60−65% peel, 30−35% internal tissues and up to 10% seeds (Crawshaw, 2004). Large quantities of citrus pulps are used for animal feeds, the majority of the processing residue is thrown out, and consequently pollutes the environment. Therefore, citrus-processing industries have been searching for applications of these by-products. Currently, almost no information is available on feeding lemon peel to rainbow trout.

Several reports have shown that the beneficial effects sour lemon peel as a feed supplement on promoting growth, health and disease resistance in fish diet (Beltran *et al*., 2017, 2019; Abdel Rahman *et al*., 2019; Harikrishnan *et al*., 2020). Due to the lack of data on lemon peel as a herbal health promoter in rainbow trout juvenile, its commercial use remains limited. Therefore, this study was designed to investigate the impacts of dietary sour lemon peel (0.5-2.5%) on hepatic enzyme activities, serum biochemical and stress status of rainbow trout juvenile.

Materials and methods

Experimental conditions

Fruit sour lemon peel was obtained from a local lemon juice market in Gorgan (Golestan Province), Iran. The products were dried at room temperature and the dried samples were powdered using an electric grinder (Pars Khazar, Tehran, Iran) for diet preparation. The experimental diet was prepared based on the previous investigation of Chekani *et al*. (2021). A commercial pellet diet

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(44% crude protein and 16% crude fat) was crushed, mixed with water to make a paste and the appropriate amount of dehydrated lemon peel powder (0, 0.5, 1.5, and 2.5%) was added. Then the mixture was passed through meat grinder to make pellet and dried for 24 h at room temperature and packed in polyethylene bags then stored at 4 °C until use. Diet without DLPP was used as control (Beltran *et al*., 2017).

A total of 300 rainbow trout was obtained from a commercial farm and raised for two weeks before the commencement of the study. After the acclimation period, the fish (mean weight of 26.8±1.9 g) were allotted into 12 tanks (700 L total volume; filled with 400 L water), with 25 fish each and fed with different level of DLPP for 45 days. During experiment period, water quality parameters were within range of the fish culture condition and temperature was 15.5±1 Celsius.

Sampling

At the end of trial, five fish were randomly selected from each tank and after anaesthetization by clove powder (100 mg/L), then blood samples were collected from the caudal vein for serum biochemical analysis. The blood samples were centrifuged at 3000 g for 15 min (Adel *et al*., 2015).

Serum biochemical parameters and Metabolic enzymes

To measure blood biochemical indicators (total protein, albumin, glucose, triglyceride, cholesterol, AST, ALP and ALT), Pars Azmoun Company kits (Tehran, Iran) were used, and then the absorptions of reacted solutions were read in a microplate reader (TECAN, Model infinite M200, Austria) and their values were reported according to the protocols.

Stress markers

The serum cortisol and glucose levels were determined by the ELISA method using a commercial kit (Pars Azmun Co, Tehran Iran).

Statistical analysis

Normality and homoscedasticity of the data were confirmed by Shapiro-Wilk and Levene

tests, respectively. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test at the five percent level of significance to detect differences among treatment means. All analyses were performed in SPSS v.22.

Results

There was no significant difference in fish growth performance and feed efficiency among treatments after 45 days.

The effects of DLPP-supplemented diets on metabolic enzymes are presented in Table 1. AST was higher in control groups and lower in 0.5 % DLPP (p<0.05). ALT did not differ between groups (p>0.05). The ALP enzyme significantly decreased in fish fed with 0.5 and 1.5% DLPP supplemented diet (p<0.05). Totally, the highest of metabolic enzymes activity were observed in control group (Table 1).

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Table 1: Metabolic enzymes activity of the fish fed with different levels dietary dehydrated lemon peel powder (DLPP) at the end of a 45-day period.

Parameter	Dietary DLPP (%)			
	Control	0.5%	1.5%	2.5%
AST (U/L)	292.3±10.7^{c}	198.6±17.5^{a}	248±19.6^{b}	255±20.7^{b}
ALT (U/L)	17.33±2.0^{a}	15±1.3^{a}	14±2.7^{a}	14.66±3.1^{a}
ALP (U/L)	706±45.3^{c}	437.3±23.6^{a}	409.5±20.8^{a}	658±54.6^{b}

Different letters in rows (mean±S.D) indicate significant differences (p<0.05) between the experimental groups.

The results of serum biochemical analysis are shown in Table 2. The results showed significant decrease in serum cholesterol level in 1.5 and 2.5% DLPP. Triglyceride level was significantly decreased in fish fed with 1.5% of DLPP compared to the other treatments (p<0.05). Protein content revealed significant (p<0.05) increases in the treated fish with 1.5% DLPP. There were no significant differences in albumin level between the fish fed DLPP and control group (p>0.05), however the highest of albumin was seen in 0.5% DLPP (Table 2).

Table 2: Biochemical blood parameters of the fish fed with different levels dietary dehydrated lemon peel powder (DLPP) at the end of a 45-day period.

Parameter	Dietary DLPP (%)			
	Control	0.5%	1.5%	2.5%
Cholesterol (mg/dl)	284±6.7^{b}	254.66±7.3^{b}	145±6.3^{a}	152.5±5.3^{a}
Triglyceride (mg/dl)	169.33±4.8^{ab}	193.33±3.8^{b}	150.33±7.4^{a}	198.5±3.6^{b}
Protein (g/dl)	4.1±0.2^{b}	4.38±0.2^{b}	3.26±0.18^{ab}	2.85±0.15^{a}
Albumin (g/dl)	1.63±0.07^{ab}	1.96±0.09^{b}	1.7±0.1^{ab}	1.5±0.12^{a}

Different letters in rows (mean±S.D) indicate significant differences (p<0.05) between the experimental groups.

Fish stress indices like cortisol and glucose did not show any significant difference between the all-dietary treatments. Nevertheless, lower value of cortisol (31.5±4.14 ng/mL) and glucose (22.5±3.2 mg/dl) were observed in 1.5% DLPP diet at the end of trial (Table 3).

Table 3: Stress status of the fish fed with different levels dietary dehydrated lemon peel powder (DLPP) at the end of a 45-day period.

Parameter	Dietary DLPP (%)			
	Control	0.5%	1.5%	2.5%
Cortisol (ng/mL)	38.5±6.25^{a}	33.1±5.29^{a}	31.5±4.14^{a}	39.7±2.15^{a}
Glucose (mg/dl)	28±2.35^{a}	24.7±5.7^{a}	22.5±3.2^{a}	26.3±5.6^{a}

Different letters in rows (mean±S.D) indicate significant differences (p<0.05) between the experimental groups

Discussion

Sustainable aquaculture is one of the most important aspects of any aquaculture industries. One approach is to use agricultural by-products as a component in aquatic feed and minimizing processing waste (Khumsrisuk *et al*., 2022). The results of

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the present study revealed that the dietary DLPP incorporation 0.5 and 1.5% feed significantly decreased the AST and ALP; indicate the normal health of liver in rainbow trout juvenile. The decrease in metabolic enzymes might be due to the antioxidant possibility DLPP compounds that can prohibit the lipid oxidation of cell membranes and inhibit the release of the transaminase enzymes. Shabana *et al*. (2019) reported that metabolic enzyme activities have remained unaffected with all concentrations of *Citrus sinensis* peel extract applications in *Catla catla*. Amiri Resketi *et al*. (2020) who noted liver enzymes of ALT and AST showed no significant differences among treatments containing essential oil, but ALP significantly increased in treatments in comparison to the control. The enhancement of hepato-protective activity in fish via by-products is fairly understood, and often times is correlated with certain phytochemicals.

In this study, the fish treatment with 1.5% DLPP lowered glucose; however, no significant difference was observed between the control and other DLPP treatments. A reduction in glucose in the DLPP-treated fish might be due to the stress reduction and several bioactive substances such as α-pinene, β-pinene (Mercier *et al*., 2009) and high levels of vitamin C (Toutou *et al*., 2018). This investigation is similar in *Oreochromis mossambicus* fed with diet citrus essential oil (Baba *et al*., 2016) and juvenile *Labeo victorianus* fed with bitter limon peel essential oil (Ngugi *et al*., 2017). Citrus lemon peels were found to significantly increase the serum glucose level in Nile tilapia in a dose-dependent manner and exhibited no effect on African catfish (Abdel Rahman *et al*., 2019). Toutou *et al*., (2018) reported that dietary lemon peel significantly decreased the glucose of Nile tilapia fed on 2% and in thin lip mullet (*Liza ramada*) fed on diets 0.5% lemon peels when compared with the control and other fishes. In gilthead seabream fed with the 1.5% DLPP the glucose level reduced at 15 days but diet supplemented with 3% DLPP do not alter after feeding for a month (Beltran *et a*l., 2019). Previous studies reported the decrease in serum glucose in Caspian white fish (*Rutilus frisii kutum*) and rainbow trout with grapefruit (*Citrus paradisi*) peel extract and lemon peel essential oil respectively (Samavat *et al*., 2019; Amiri Resketi *et al*., 2020). These different results might be due to different factors such as experimental situation, fish species and culture period.

The present study revealed that supplementation of diet with DLPP did not significantly affect on serum cortisol in rainbow trout juvenile which is in agreement with the result of the study by Abdel Rahman *et al*. (2019) who studied the effect of dehydrated lemon peel on Nile tilapia and African catfish fed on dehydrated lemon peel and Amiri Resketi *et al*. (2020) who investigated the effect of lemon peel essential oil on rainbow trout. The mitigated level of cortisol in the present study is related to active compounds (Mercier *et al*., 2009) in lemon peel. Ngugi *et al*. (2017) observed that dietary administration of

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C. limon peel essential oil in *L. victorianus* decreased the cortisol level.

The present study revealed that in the groups fed 1.5% DLPP supplemented diets the cholesterol and triglyceride levels decreased. According to the results obtained by Toutou *et al*. (2018), the Nile tilapia fed 2% dietary lemon peel had the lowest cholesterol and triglyceride, but the highest value was obtained in 2% DLPP in thin lip mullet (*Liza ramada*). Similar to our result, Baba *et al*. (2016) documented that a dietary essential oil of *C. limon* administration significantly decreased cholesterol and triglyceride levels in Mozambique tilapia (*Oreochromis mossambicus*). Likewise, administration essential oil extract of *C. limon* fruit peels from 2% to 5% decreased triglyceride and cholesterol in Juvenile *Labeo victorianus* fingerlings (Ngugi *et al*., 2017). A study conducted by Samavat *et al*. (2019) also show that dietary supplementation of grapefruit (*Citrus paradisi*) peel extract resulted significant decreases in serum cholesterol level in Caspian white fish. Amiri Resketi *et al*. (2020) reported that the triglyceride level was not significantly affected by dietary lemon peel essential oil, but cholesterol was significantly increased compared to the control. The reduced level of cholesterol supports the possibilities of the inhibition of de novo cholesterol biosynthesis by *C. limon* peel (Ngugi *et al*., 2017) and providing the healthier status of liver (Terpstra *et al*., 2002).

In current study, serum protein increased in fish fed with 0.5% DLPP in comparison with the other groups, but there were no significant differences in albumin level between the fish fed DLPP and control group. The increase in serum protein content might be correlated with an increase of proteins like serum lysozyme, complement component, acute phase proteins, cytokines, lectins and bactericidal peptides (Yilmaz *et al.*, 2014). Ngugi *et al*. (2017) indicated that total protein and albumin in blood of Juvenile *Labeo victorianus* fingerlings were higher with increasing dietary *C. limon* essential oil up to 5% level. Similar to our finding, Baba *et al*. (2016) showed that addition of 0.5 % *C. limon* essential oil in diet of Mozambique tilapia increased serum protein level while the albumin level did not differ among the groups. Acar *et al*. (2015) observed a significant increase in total protein in tilapia fed with different concentration of *Citrus sinensis* essential oil. Also, supplementation of diet with lemon peels essential oil increased in protein and albumin in rainbow trout (Amiri Resketi *et al*., 2020). Different values in biochemical variables depends on several factors, e.g., drying method, dose of the used, concentration and profile of bioactive components, physiological state of fish, background diet and housing conditions.

In conclusion, the results of this study demonstrated that Supplementing rainbow trout diet with 0.5 and 1.5% DLPP could improve the serum hepatic health, biochemical parameters and reduced stress responses.

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