EFFECTS OF ETHANOLIC POLLEN EXTRACT IN VARIOUS CONCENTRATIONS ON TOTAL RNA AND TOTAL PROTEIN LEVELS IN DIFFERENT TISSUES OF RAINBOW TROUT (*ONCORHYNCHUS MYKİSS*)

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Abstract: In this study, the effects of ethanolic pollen extract in various concentrations (0.5, 2.5, 5, 10, 20 and 30 ppm) on total RNA and total protein levels in different tissues of rainbow trout have been investigated. Pollen extract in various concentrations was administered to aquarium which habitat of fish for 96 h. Total RNA and total protein levels were analyzed in muscle, gill, liver, spleen, heart and brain of rainbow trout (*Oncorhynchus mykiss*). Total RNA levels in gill, liver and heart tissues of various concentration groups (0.5, 2.5, 5, 10, 20 and 30 ppm) increased (P<0.05) compared to control group. The highest value of total RNA (P<0.05) occured in spleen and brain tissues of 20 and 30 ppm concentration groups. Total RNA level increased in muscle tissue of 30 ppm concentration group compared to control group (P<0.05). The highest values of total protein levels have been occured (P<0.05) in all tissues of 10 and 20 ppm groups compared with control group. Changes in total protein levels in 0.5 ppm concentration groups of all tissues have not been determined compared to control group (P>0.05). As a result, total RNA and total protein levels depend on concentrations of pollen in some tissues of fish.

Keywords: pollen, total protein, total RNA, Rainbow trout

Rezumat. Efecte ale extractului etanolic de polen în concentratii variate asupra nivelurilor de ARN total şi proteină totală, în diferite ţesuturi ale păstrăvului curcubeu (*Oncorhynchus mykiss).* In prezentul studiu au fost investigate efecte ale extractului etanolic de polen în concentraţii variate (0,5; 2,5; 5; 10; 20 şi 30 ppm) asupra nivelurilor de ARN total şi proteină totală în diferite ţesuturi ale păstrăvului curcubeu. Extract de polen, în diferite concentraţii, a fost administrat în acvariu, în care au stat peşti timp de 96 ore. Nivelurile de ARN total şi de proteină totală au fost analizate din muşchi, branhii, ficat, splina, inima şi encefal de păstrăv curcubeu (*Oncorhynchus mykiss*). Nivelurile de ARN total din ţesuturi branhiale, hepatice, splenice, cardiace şi encefalice provenite de la grupurilor tratate cu concentraţii variate (0,5; 2,5; 5; 10; 20 şi 30 ppm) au crescut (P<0,05) în comparaţie cu grupul de control. Cea mai mare valoare a ARN-ului total s-a înregistrat în splina şi encefalul prelevate de la grupurilor tratate cu concentraţii de 20 şi 30 ppm. Nivelul de ARN total dn ţesutul muscular prelevat de la grupul tratat cu concentratie de 30 ppm a crescut în comparatie cu grupul de control (P<0.05). Cele mai mari valori ale nivelurilot de de proteina totală au fost inregistrate în toate ţesuturile grupurilor tratate cu 10 şi 20 ppm, în comparaţie cu grupul de control. Nu au fost înregistrate modificări în nivelurile proteinei totale din ţesuturile prelevate de la grupul tratat cu concentraţia de 0.5 ppm, in comparaţie cu grupul de control (P>0.05). Prin urmare, nivelurile de ARN total şi de proteină totală depind de concentraţiile de polen în unele ţesuturi la peşti.

Cuvinte cheie: polen, proteina totala, ARN total, pastravul curcubeu

Introduction

Most of investigations are releated to antioxidant capacities of different nutritional products nowadays. The antioxidative properties of phenolic compounds of natural products have been observed (Talas & Gulhan, 2009). Honeybee products, especially rich in flavonoids, have been the focus of investigations. Among them, special attention should be paid to the floral pollen used for many years as a beneficial dietary supplement (Leja *et al.*, 2007). Bee pollens are the male generative cells gathered by honeybees from flower stamens. It provides nutrition through its remarkable quantity of proteins, sterols, fatty acids, vitamins, carbon hydrates, lipids, vitamins, ashes, minerals, phenolic compounds and flavonoids which are regarded as protective agent (Talas & Gulhan, 2013; Gulhan *et al.*, 2014).

Fish are one of the most important aquatic organisms which can produce significant sources of protein for human nutrition (Duran & Talas, 2009; Gulhan *et al.*, 2012). Fish are very sensitive against changes in their environment. Fish are commonly used to estimate the influences of environmental compounds due to the sensitivity of their biochemical parameters under of environmental conditions (Talas *et al.*, 2014). As a result of these changes may be affected the certain tissues and organs of fish, including muscle, gill, liver, spleen, heart and brain.

Fish muscle has an important role in the human diet. Stress and excessive muscle activity lead to insufficient amount of oxygen (Gulhan *et al.*, 2012). Gills generally absorb water-soluble foreign compounds (Fanta *et al.*, 2003). Fish liver is the primary organ associated to the biotransformation of organic materials. This organ is very sensitive against organic and inorganic agents which is releated to the environment itself and other organs (Stori *et al.*, 2014). Spleen is erythropoietic tissue involves in the synthesis of new erythrocytes and reservoir in primary hemopoietic organs. Spleen is the unique organ in fish to trap antigens (Balamurugan *et al.*, 2012). Contraction and relaxation in the working of fish heart are a result of the complex interaction of many individual cells connected together by specialized adhesion structures. The heart allows generating pressure to pump blood around the body. This organ is important for adaptation to environmental conditions such as fluctuating, temperatures, oxygen and pH (Galli & Richards, 2012). Due to the brain being the most shielded part of the body and the lipophilic structure of the brain does not allow some materials to pass over cell membranes (Gulhan *et al.*, 2014).

Fish are an important aquatic organism. Fish products are an important source of protein for human consumption (Selamoglu *et al.*, 2012). Long chain polyunsaturated fatty acids (PUFA) are conditionally essential nutrients for adequate growth, development and function in humans (Gil *et al.*, 2012). Among them, omega-3 PUFA (ω3 PUFA) have gained popularity due to their various health promoting and diseases preventing attributes (Wang *et al.*, 2012).

Assay of total RNA levels was done for deriving introductory information about the total protein amount in organism. The study on total RNA levels is also significant since it might provide introductory knowledge about total protein amount. There is no sufficient evidence about the modulating role of pollen on total protein and total RNA levels after pollen administration in fish. The present study was designed to test the impact of ethanolic pollen extracts in different concentrations on total protein and total RNA levels in muscle, gill, liver, spleen, heart and brain of fish.

The comparing the total protein amounts of fish with various concentrations at supplement diets helps to understand their metabolic and physiological distinctions and also

environmental interactions of rainbow trout.

Our study aimed to occur the effects of various concentrations (0.5, 2.5, 5, 10, 20 and 30 ppm) of ethanolic pollen extract on total protein and total RNA levels in muscle, gill, liver, spleen, heart and brain tissues of rainbow trout (*Oncorhynchus mykiss*).

Material and Methods

Animals and experimental design. Rainbow trouts (*Oncorhynchus mykiss*) with average weight of 248.54±5.12 g were obtained from Camardi, Ecemis fish farm in Nigde, Turkey. Then they were transferred to research station in Nigde University under optimum conditions with the dimension of 8x5x1.5 m and acclimated for 15 days. They were fed with commercial food once daily. Physical and chemical parameters of water are shown in table 1. At the present study, seven experimental groups, each consisting of eight animals, were used. It has four replicates including 7 fish each. Randomly, we chosen 2 fish from every tank. As a result, each experimental group including totally 8 animals with four replicate. The fish administered to 0.5 ppm pollen extract as group I, 2.5 ppm as group II, 5 ppm as group III, 10 ppm as group IV, 20 ppm as group V 30 ppm as VI and untreated fish as control group were used for 96 h. Then, they were sacrificed in accordance with the guidelines for approved by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

Table 1. Amount of physical and chemical parameters of water during the present study.

Parameters	Before treatment	After treatment
Dissolved oxygen (ppm)	7.6 ± 0.6	7.4 ± 0.3
Chemical oxygen demand (ppm)	13.1 ± 0.4	15.5 ± 0.8
Suspended solids (ppm)	37.6 ± 1.5	41.1 ± 1.2
Calcium (ppm)	132.0 ± 1.8	109.1 ± 1.5
Sodium (ppm)	24.4 ± 0.4	17.7 ± 0.3
Chloride (ppm)	15.0±1.2	16.0±1.8
Total nitrogen (ppm)	5.3 ± 0.5	6.2 ± 0.7
Hardness ($CaCO_3$) (ppm)	179.3 ± 3.6	163.2 ± 2.3
Temperature (^oC)	12.5 ± 1.6	11 ± 0.3
pH	7.6 ± 0.1	7.6 ± 0.1

Preparation of pollen extractive solution. Pollen was obtained from a farm at village Kocaavsar in Balikesir, Turkey and diluted to 30% in ethanol. It was kept in dark at room temperature and moderately shaken for one day. Afterward, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until used (Marghitas *et al.*, 2009).

Preparation of tissues for biochemical analyses. After application for 96 hours, fish were anaesthetised with clove oil (Mylonas *et al.*, 2005). Muscle, gill, liver, spleen, heart and brain tissues of fish were removed and stored at -80 °C until used. Tissues were weighed and then homogenized in 100 mL of 2 mM phosphate buffer, pH 7.4. Samples were centrifuged at 12,000 g for 10 min at 4 °C and then supernatants were kept in the deep freeze at -80 °C until analysed. Supernatants were used for determination of total protein and total RNA levels.

Protein Assay. Supernatants of fish tissues were used for determination of total protein. Total protein was quantified by the colorimetric method of Lowry et al. using BSA as the standard (Lowry *et al.*, 1951).

Measurement of Total RNA Levels. Three steps were performed on the supernatants of tissues to measure total RNA levels (Chomzynski & Sacchi, 1987). These stages are (i) extraction, (ii) precipitation and washing with ethanol later, and (iii) resolution in double-distilled water. After the three stages, total RNA levels were quantified spectrophotometrically at 280 nm (Chomzynski & Sacchi, 1987).

Statistical analysis. Because parametric test hypotheses were implemented in the data evaluation by uploading the data, which were obtained from our study, on SPSS ver. 22.0, (Kolmogorof-Simirnov) the variance analysis, Tukey's test, Anova analysis and Correlation analysis were applied and the error level was considered as 0.05.

Results and Discussion

The effects of pollen extracts in various concentrations on total RNA and total protein levels in muscle, gill, liver, spleen, heart and brain tissues of rainbow trout (*Oncorhynchus mykiss*) have been showed in table 2 and 3. Total RNA levels in muscle, spleen and brain tissues of fish administered to 0.5 ppm pollen extract did not change ($P>0.05$) compared to control group (Table 2), but there were statistically increases ($P<0.05$) in total RNA levels levels of all of tissues of 30 ppm pollen groups (Table 2).

Total protein levels of all tissues with 0.5 ppm pollen administration were stable ($P>0.05$) compared with control group (Table 3). But, total protein levels in all of tissues of 20 and 30 ppm pollen groups significantly increased compared to control group ($P<0.05$) (Table 3).

In the present study, there were statistically significant ($P<0.05$) increases in total RNA levels of gill, liver and heart tissues of all experimental groups applied to all concentrations (0.5, 2.5, 5, 10, 20 and 30 ppm) of pollen extract compared to control group. Generally, the highest value of total RNA ($P<0.05$) occured in 20 and 30 ppm concentration groups of tissues.

Our data show a significant change in total protein levels among different pollen concentration groups. The total RNA levels in fish tissues also increased depending on concentration of pollen. Synthesis of all proteins depends on translation of RNA. Changes in the levels of total protein may be parallel with changes in the levels of total RNA recorded. The total RNA levels may act as an indicator of the total protein levels. Increasing the level of RNA involved in these tissues of rainbow trout (*Oncorhynchus mykiss*) shows interaction at the transcriptional level. Increasing concentrations of pollen may change the transcriptional case in the fish tissues.

Conclusions

This work was determine the effective concentration of pollen on biochemical parameters in tissues of aquatic animals. Thus, this study aimed to determine the effective concentrations of pollen extract on biochemical parameters in muscle, gill, liver, spleen, heart and brain of rainbow trout. As a result, the present study shows that pollen causes understanding of the interactions at the transcriptional and translational levels in rainbow trouts. For the first time, the present work investigates the effects and useful concentration of pollen on biochemical parameters in some tissues of fish. The results suggest that pollen may possess transcriptional and translational expressions that could influence serum total RNA and total protein levels in fish. The data of this study will shed light on new researchs in the future and contribute to the scientific literature.

Table 2. Changes in total RNA levels with ethanolic pollen extract in various concentrations in different tissues of rainbow trout (*Oncorhynchus mykiss*).
The mean difference is significant at the 0,05 level. [a, b, c, d] is statistically different.

Groups / Tissues	Mean±SD (µg/mL)							F	Significant
	Control	0.5 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm	30 ppm		
Muscle	36.01±9.26 [b]	37.65±7.41[b]	40.25±5.79[b]	43.12±4.01[b]	54.97±3.31[b]	61.54±5.84[b]	127.03±80.49[a]	6.48	0.001
Gill	32.07±5.85[c]	54.07±6.50[b]	49.29±8.37[b]	54.45±11.56[b]	67.65±5.44[a]	69.61±12.71[a]	80.81±2.61[a]	22.28	0.001
Liver	74.74±31.96[c]	79.68±7.87[b]	91.22±5.93[b]	90.92±7.76[b]	100.30±3.34[a]	142.29±19.98[a]	122.72±9.59[a]	14.63	0.001
Spleen	38.69±16.81[c]	32.30±12.89[c]	40.00±2.48[c]	53.10±4.61[b]	61.51±5.04[b]	307.78±39.20[a]	233.29±30.46[a]	3.42	0.009
Heart	26.55±16.73[c]	45.81±9.18[b]	55.56±3.42[a]	40.24±14.69[b]	49.58±5.38[b]	54.18±9.25[a]	68.55±10.74[a]	11.47	0.001
Brain	17.88±9.39[b]	26.27±10.15[b]	25.86±5.58[b]	29.04±7.60[b]	43.79±6.22[b]	53.71±6.83[a]	88.27±51.16[a]	8.27	0.001

Table 3. Changes in total protein levels with ethanolic pollen extract in various concentrations in different tissues of rainbow trout (*Oncorhynchus mykiss*).
The mean difference is significant at the 0,05 level. [a, b, c, d] is statistically different.

Groups / Tissues	Mean±SD (mg/mL)							F	Significant
	Control	0.5 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm	30 ppm		
Muscle	53.34±6.50[b]	77.29±32.56[b]	147.38±11.22[a]	123.88±26.94[a]	104.56±20.10[a]	96.17±47.31[a]	145.97±80.93[a]	4.59	0.002
Gill	55.20±18.56[b]	47.90±6.83[b]	82.38±30.05[b]	92.88±21.07[b]	85.24±9.18[b]	113.77±24.77[a]	129.01±83.24[a]	3.76	0.005
Liver	68.27±17.93[b]	54.18±18.68[b]	65.98±12.60[b]	84.38±7.52[b]	94.05±12.33[a]	104.26±33.02[a]	130.70±35.46[a]	8.53	0.001
Spleen	40.14±12.82[b]	38.52±12.94[b]	48.95±11.71[b]	59.17±7.85[b]	69.45±5.82[a]	73.43±4.19[a]	90.02±9.09[a]	22.62	0.001
Heart	80.40±10.49[b]	71.05±15.13[b]	62.72±16.05[b]	99.76±14.51[b]	93.68±12.66[b]	129.70±21.08[a]	122.94±9.98[a]	17.74	0.001
Brain	45.19±10.25[c]	67.91±25.91[c]	128.43±5.95[b]	131.68±13.63[b]	136.79±23.52[b]	139.48±10.77[a]	165.15±47.41[a]	19.90	0.001

In conclusion, protein levels in fish tissues were related to concentrations of pollen extract. The total RNA and protein levels in fish are also directly related to additive foods.

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