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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Biochemical changes in muscle and gill tissues of rainbow trout treated with various concentrations of pollen extract

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Abstract

The aim of this study was to determine the effectiveness of pollen extract on biochemical parameters in muscle and gill tissues of rainbow trout. Various concentrations of the pollen extract (0.5, 2.5, 5, 10, 20 and 30 ppm) for 96 h was applied on muscle and gills of forty nine rainbow trout (*Oncorhynchus mykiss*). The malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), total free sulfhydryl groups were determined. MDA level decreased ($P<0.05$) at the dose rate of 10-30 ppm concentration in muscle while in gills the level of 10 ppm was the most effective. The dose rate of 10 ppm was effective in TAS, TOS and OSI for muscle and gill tissues. The highest value of total free sulfhydryl groups was found at the dose rare of 5 and 10 ppm in muscle and tissues respectively. It is concluded that pollen extract on muscle and gill tissues of fish is effective in prevention of oxidative stress.

Keywords: Antioxidant; gill; muscle; pollen; rainbow trout

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Introduction

Fish are one of the most important aquatic organisms which can produce significant sources of protein for human nutrition (Duran and Talas, 2009). The significance of long-chain polyunsaturated fatty acids has gained attention because of their preservative effect on human cardiovascular damage (Wang et al., 2012). Physiological stress such as environmental conditions can cause oxidative stress, reflected by an increased production of reactive oxygen species (ROS) and decreased in antioxidants that play a fundamental role in defence system (Sahin et al., 2014). Recently, most of investigations have been concerned over the various nutritional products due to their antioxidant potential to prevent or treat the diseases of animals (Marghitas et al., 2009; Li et al., 2009). Natural nutritional antioxidants including the content of low-molecular antioxidants such as carotenoids, tocopherols, ascorbic acid, phenolic substances have generally contributed to the activities of antioxidant enzymes (Gulhan et al., 2012).

Pollen is one of these natural antioxidant agents. Bee pollens are the male generative cells gathered by honeybees from flower stamens and provide plenty of phenolic compounds (LeBlanc et al., 2009). Honey bee-collected pollen is an apicultural product which is composed of nutritionally valuable substances and includes significant amounts of polyphenolic compounds, mainly flavonoids, which may act as effective antioxidants (Marghitas et al., 2009). Malondialdehyde (MDA), which is an indicator of lipid peroxidation was used as indicators of local tissue injury. Lipid peroxides can change properties of biological membranes, resulting in free radical damage (Sanz et al., 2013). From the changes of total sulfhydryl

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groups (T-SH) in proteins, we can understand more about the conformation change and formation of disulfide bonds (Ko et al., 2007). The contents of phenolic compounds in pollen such as phenolic acid, flavonoids and tannins are thought to be an important source of the antioxidant capacity of foods (Marghitas et al., 2009). After some antioxidants treatments, the assays of total antioxidant status (TAS) and total oxidant status (TOS) can reflect the biochemical changes in tissues (Sekhon-Loodu et al., 2013).

In the present study, the effects of pollen extract in different concentrations were investigated by analysis of biochemical parameters (TAS, TOS, OSI, Total Sulfhydryl Groups and MDA) in muscle and gill tissues of fish.

Materials and Methods

Animals and experimental design

Forty nine rainbow trout (*Oncorhynchus mykiss*) with average weight of 248.54±5.12 g were obtained from Camardi, Ecemis fish farms, Nigde, Turkey. They were transferred to research laboratory in Nigde University under optimum conditions, and distributed to seven stock ponds (7 fish each) with the dimension of 8×5×1.5 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.

Table 1: Pphysical and chemical parameters of water during of 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 Kocaavsar in Balikesir, Turkey and diluted to 30% in ethanol, kept in dark, at room temperature and moderately shaken for several minutes every day. Afterward, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until use (Marghitas et al., 2009).

Experimental design

Pollen extract at the rate of 0.5, 2.5, 5, 10, 20 and 30 was applied on fish for 96 h. One group served as a control (untreated). After treatment, fish were sacrificed in accordance with the guidelines approved by the Committee of Animal Experiments at Cumhuriy et University, Sivas, Turkey.

Preparation of tissues for biochemical analysis

After application for 96 h, fish were anaesthetised with clove oil at the rate of 40 mg/l (Mylonas et al., 2005). Muscle and gill tissues of fish were removed and stored at -80°C until used. The tissues were separated into two parts for determination of MDA levels and the other biochemical parameters. Tissues were weighed and then homogenized in 2 mM phosphate buffer, pH 7.4. Samples were centrifuged at 12,000g for 10 min at 4°C and supernatants, if not used for enzyme assay immediately, were kept in the deep freeze at -80°C. Supernatants were used for determination of TAS, TOS, OSI and total sulfhydryl groups. The second part of tissues was used for MDA analysis. Tissues were washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for MDA.

Malondyaldehyde (MDA) levels

The malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by using proper method (Esterbauer and Cheeseman, 1990).

Total Antioxidant Status (TAS)

Total antioxidant status in tissue was tested following the method of Erel (2004). The test has very sensitive values of <3%. Result were expressed as mmol Trolox equiv./l (Erel, 2004).

Total Oxidant Status (TOS)

TOS in tissue was analysed using automatic measurement method developed by Erel (2005). The test was calibrated with hydrogen peroxide and the obtained results were expressed in terms of μmol H_2O_2 Equiv./l (Erel, 2005).

Oxidative Stress Index (OSI)

The ratio of TOS to TAS was measured to be the OSI (Erel, 2004; 2005). The OSI level was calculated according to the following formula:

OSI (arbitrary unit) = TOS/TAS x100

Total free sulfhydryl groups

Free sulfhydryl groups of tissue patterns were determined according to the method of Ellman followed by Hu (Ellman, 1959; Hu et al., 1993). The concentration of sulfhydryl groups was calculated using reduced glutathione as free sulfhydryl group standard and the result was expressed as mmol/mg protein (Sanz et al., 2013).

Statistical analysis

Data were tested by SPSS 16.0 for Windows using nonparametric Kruskal-Wallis test. Differences between

Table 2: Changes in the biochemical parameters in muscle tissues of rainbow trout treated with various concentrations of pollen

Parameters	Groups and Concentrations						
	Control	0.5 ppm (Group I)	2.5 ppm (Group II)	5 ppm (Group III)	10 ppm (Group IV)	20 ppm (Group V)	30 ppm (Group VI)
MDA (nmol/g wet tissue)	16.99±0.77^{a}	11.58±0.68^{b}	10.20±0.93^{b}	9.61±0.99^{b}	7.57±1.01^{c}	7.34±0.51^{c}	7.72±0.50^{c}
TAS (mmol Trolox equivalent/g protein)	1.15±0.07^{c}	1.27±0.07^{c}	1.58±0.04^{b}	1.61±0.10^{b}	1.87±0.06^{a}	1.48±0.12^{b}	1.53±.0.09^{b}
TOS (µmol H_2O_2 equivalent/l)	2.74±0.18^{a}	2.32±0.17^{b}	2.27±0.11^{b}	2.21±0.06^{b}	1.98±0.16^{c}	2.02±0.06^{c}	2.35±0.09^{b}
OSI (arbitrary units)	0.24±0.02^{a}	0.18±0.01^{b}	0.14±0.01^{b}	0.13±0.01^{b}	0.10±0.02^{c}	0.13±0.02^{b}	0.15±0.03^{b}
Total free sulfhydryl group (mmol/mg of protein)	0.69±0.39^{c}	1.43±0.16^{b}	1.65±0.12^{b}	2.05±0.08^{a}	1.43±0.17^{b}	1.60±0.09^{b}	1.34±0.06^{b}

All data points are the average of n= 7 with ± SD. $^{a.b.c}$ statistically significant (P<0.05)

Table 3: Changes in biochemical parameters in gill tissues of rainbow trout treated with various concentrations of pollen

Parameters	Groups and Concentrations						
	Control	0.5 ppm (Group I)	2.5 ppm (Group II)	5 ppm (Group III)	10 ppm (Group IV)	20 ppm (Group V)	30 ppm (Group VI)
MDA(nmol/g wet tissue)	9.80±3.99^{a}	4.28±1.66^{b}	5.77±6.24^{b}	4.68±0.68^{b}	2.97±0.75^{c}	4.10±0.90^{b}	5.17±1.09^{b}
TAS (mmol Trolox equivalent/g protein)	1.05±0.08^{c}	1.36±0.10^{c}	1.79±0.09^{c}	1.69±0.11^{b}	2.01±0.07^{a}	1.96±0.07^{a}	1.65±0.07^{b}
TOS (µmol H2O2 (equivalent/l)	1.85±0.12^{a}	1.78±0.10^{a}	1.58±0.06^{b}	1.55±0.11^{b}	1.16±0.04^{c}	1.21±0.10^{c}	1.55±0.02^{b}
OSI	0.17±0.03^{a}	0.13±0.01^{b}	0.08±0.07^{b}	0.09±0.05^{b}	0.05±0.02^{c}	0.06±0.01^{c}	0.09±0.05^{b}
Total free sulfhydryl group (mmol/mg of protein)	0.10±0.06^{c}	0.87±0.14^{b}	0.38±0.05^{c}	0.97±0.02^{b}	1.29±0.16^{a}	0.81±0.12^{b}	0.85±0.05^{b}

ranks were determined using Mann-Whitney test in which the significance level was defined as P<0.05.

Results

Changes in biochemical parameters in muscle tissue of control and treated groups are given in Table 2. Significant reduction in MDA concentration was found at the level of 10, 20 and 30 ppm compared to other groups. TAS level was significantly high in group treated with 10 ppm pollen grain. TOS level was significantly low in group at the level of 10 and 20 ppm. Similarly, OSI was significantly low at the level of 10 ppm. Total free sulfhydryl group was recorded significantly high at the dose rate of 5 ppm compared to the control and other treatments.

Biochemical changes in gill tissue are given in Table 3. The results showed that MDA level was significantly high at the dose rate of 10 ppm while TAS was significantly high at the dose level of 10 and 20 ppm. TOS and OSI were significantly low at the dose level of 10 and 20 ppm. Total free sulfhydryl group was significantly high in group treated with 10 ppm dose rate.

Discussion

Many studies have reported the importance of natural antioxidants in human health in recent years. Bee pollen is extremely rich in natural antioxidants such aspolyphenols and flavonoids therefore it may show various pharmacological effects and increase the capacity of the cells which neutralize oxidative stress and (Kroyer and Hegedus, 2001). In the present study, significant reduction in MDA levels of muscle and gill tissues in all experimental groups was observed. The antioxidant activity of honeybee-collected pollen has been recognised as a free radical scavenger and a lipid peroxidation inhibitor (Almaraz-Abarca et al., 2004).

Hamre et al. (2010) reported significant effects of various natural antioxidants (rosemary extract, crystalline ascorbic, acid tocopherol mix, spermine etc.) in experimental fish feed and concluded that they have potent antioxidant properties. Sahin et al. (2014) suggested that supplementation of different doses of lycopene caused a dose dependent decrease in MDA as well as increase in antioxidant enzymes (GSH-Px, SOD and CAT) in the liver of fish. In another study, a similar effect on MDA level in rats was observed in response to pollen extract treatment (Eraslan et al., 2009). Other researchers have found that there is a positive correlation between proportional reduction of MDA values with the amount of flavonoids (Sahin et al., 2014; Bacchetta et al., 2014). Talas and Gulhan (2009) determined positive effect on biochemical and haematological parameters in blood of rainbow trout treated with 0.01 g/l concentrations of propolis for 96 h. It may be hypothesized that pollen may possess antioxidant properties that could influence oxidant and antioxidant balance. Scavenging free radicals and

inhibition of lipid peroxidation are related to flavonoid compounds of pollen (Saric et al., 2009). In the current study, 10 ppm extract of pollen is more effective on TAS levels of muscle and gill tissues compared to the other concentrations. Chenga et al. (2013) investigated the effects of pollen extract on DNA damage and oxidative stress and concluded that pollen extract has radical scavenging effect to protect DNA due to the presence of fenolic and flavonoid compounds. In another investigation, lipoic acid (70 mg/kg of body mass) enhanced TAS levels in gills, brain, muscle and liver of *Corydoras* P*aleatus* (Monserrat et al., 2008).

Structural degradation of proteins is related to the formation of disulfide bonds and conformational changes in proteins, resulting in an increase in aggregated β-sheet structure and a decrease in α-helical structure of proteins (Carton et al., 2009). This case includes the changes in reactive groups, such as loss of hydrophilic surface, exposure of hydrophobic areas and sulfhydryl groups that are buried or blocked in native proteins (Hsu et al., 2007). Total free sulfhydryl levels in muscle tissue at the rate of 5 and 10 ppm pollen respectively were increased compared to control group. Sulfhydryl groups are natural antioxidants which prevent oxidants. Sulfhydryl groups have antioxidant capacity that protects cells from oxidative stress caused by free radicals (Manda et al., 2010).

This work is the first report to determine the effectiveness of pollen extract on biochemical parameters in muscle and gill tissues of aquatic animals. The antioxidant properties of pollen may prolong the physiological and metabolic functions of some aquatic living organisms and prevent the development of lipid per oxidation in muscle and gill tissues of fish.

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References

Almaraz-Abarca, N., Campos, M.G., Avila-Reyes, A., Naranjo-Jimenez, N., Herrerra- Corral, J. and Gonzales-Valdez, L.S. 2004. Variability of antioxidant activity among honeybee-collected pollen of different botanical origin. *Intersciencia,* 29: 574-578.

Bacchetta, C., Rossia, A., Ale, A., Campana, M., Parma, M.J. and Cazenavea, J. 2014. Instituto Nacional de Limnología (INALI-CONICET-UNL), Ciudad PEP. Combined toxicological effects of pesticides: A fish multi-biomarkerapproach. *Ecology Indicators,* 36: 532-538.

Carton, I., Bocker, U., Ofstad, R., Sorhem, O. and Kohler, A. 2009. Monitoring secondary structural changes in salted and smoked salmon muscle myofiber proteins by FT-IR microspectroscopy. *Food Chemistry,* 57: 3563-3570.

Chenga, N., Wang, Y., Gao, H., Yuanb, J., Fengc, F., Caoa, W. and Zhenga, J. 2013. Protective effect of extract of Crataegus pinnatifida pollen on DNA damage response to oxidative stress. *Food Chemistry Toxicology*, 59: 709-714.

Duran, A. and Talas, Z.S. 2009. Biochemical changes and sensory assessment on tissues of carp (*Cyprinus carpio*, Linnaeus 1758) during sale conditions. *Fish* P*hysiology and Biochemistry*, 35: 709-714.

Ellman, G.L. 1959. Tissue sulfhydryl groups. *Archive of Biochemistry and Biophysics*, 82: 70-77.

Eraslan, G., Kanbur, M., Silici, S., Liman, B.C., Altınordulu, S. and Soyer Sarıca, Z. 2009. Evaluation of protective effect of bee pollen against propoxur toxicity in rat. *Ecotoxicology and Environmental Safety*, 72: 931-937.

Erel, O. A. 2004. Novel automated method to measure total antioxidant response against potent free radical reactions. *Clinical Biochemistry,* 37: 112-9.

Erel, O. 2005. A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry,* 38: 1103-11.

Esterbauer, H. and Cheeseman, K.H. 1990. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymology*, 186: 407-421.

Gulhan, M.F., Duran, A., Talas, Z.S, Kakoolaki, S. and Mansouri, S.M. 2012. Effects of propolis on microbiologic and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) after exposure to the pesticide. *Iranian Journal of Fish Science,* 11: 490-503.

Hamre, K., Kolas, K. and Sandnes, K. 2010. Protection of fish feed, made directly from marine raw materials, with natural antioxidants. *Food Chemistry*, 119: 270-278.

Hsu, K.C., Hwang, J.S., Yu, C.C. and Jao, C.L. 2007. Changes in conformation and in sulfhydryl groups of actomyosin of tilapia (*Orechromis niloticus*) on hydrostatic pressure treatment. *Food Chemistry*, 103: 560-564.

Hu, M.L., Louie, S., Cross, C.E., Motchnik, P. and Halliwell, B. 1993. Antioxidant protection against hypochlorous acid in human plasma. *Journal of Laboratory Clinical Medicine*, 121: 257-62.

Ko, W.C., Yu, C.C. and Hsu, K.C. 2007.Changes in conformation and sulfhydryl groups of tilapia actomyosin by thermal treatment. *LWT-Food Science Technology,* 40: 1316-1320.

Kroyer, G. and Hegedus, N. 2001. Evaluation of bioactive properties of pollenextracts as functional dietary food supplement. *Innovative Food Science & Emerging Technologies,* 2: 171-174.

LeBlanc, B.W., Davis, O.K., Boue, S., De Lucca, A. and Deeby, T. 2009. Antioxidant activity of Sonoran Desert bee pollen. *Food Chemistry*, 115: 1299-1305.

Li, F., Yuan, Q. and Rashid, F. 2009. Isolation, purification and immunobiological activity of a new water-soluble bee pollen polysaccharide from *Crataegus* P*innatifida* Bge. *Carbohydrate* P*olymers*, 78: 80-88.

Manda, K.R., Adams, C. and Ercal, N. 2010. Biologically important thiol in aqueous extract of spices and evaluation of their in vitro antioxidant properties. *Food Chemistry,* 118: 589-593.

Marghitas, L.A., Stanciu, O.G., Dezmirean, D.S., Bobis, O., Popescu, O., Bogdanov, S. and Campos, M.G. 2009. In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. *Food Chemistry*, 115: 878-883.

Monserrat, J.M., Lima, J.V., Ferreira, J.L.R., Acosta, D., Garcia, M.L., Ramos, P.B., Moraes, T.B., Santos, L.C. and Amado, L.L. 2008. Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras* P*aleatus* (Callychthyidae). *Comparative Biochemistry* P*hysiology Part C*, 148: 287-292.

Mylonas C.C., Cardinaletti, G., Sigelaki, I. and Polzonetti-Magni, A. 2005. Comparative efficacy ofclove oil and 2-phenoxyethanolas anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*SParus aurata*) at different temperatures. *Aquaculture,* 246: 467-481.

Sahin, K., Yazlak, H., Orhan, C., Tuzcu, M., Akdemir, F. and Sahin, N. 2014. The effect of lycopene on antioxidant status in rainbow trout (*Oncorhynchus mykiss*) reared under high stocking density. *Aquaculture*, 418-419, 132-138.

Sanz, A., Trenzado, C.E., Castro, H.B., López-Rodríguez, M.J. and Figueroa, J.M.T. 2013. Relationship between brain and liver oxidative state and maximum lifespan potential of different fish species. *Comparative Biochemistry and Physiology Part A*, 165: 358-364.

Saric, A., Balog T., Sobocanec, S., Kusic B., Sverko, V., Rusak, G., Likic, S., Bubalo, D., Pinto, B., Reali, D. and Marotti, T. 2009. Antioxidant effects of flavonoid from Croatian Cystus incanus L. rich bee pollen. *Food Chemistry Toxicology,* 47: 547-554.

Sekhon-Loodu, S., Warnakulasuriya, S.N., Rupasinghe, V.H.P. and Shahidi, F. 2013. Antioxidant ability of fractionated apple peel phenolics to inhibit fish oil oxidation. *Food Chemistry*, 140: 189-196.

Talas, Z.S. and Gulhan, M.F. 2009. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout **(**Oncorhynchus mykiss). *Ecotoxicology and Environmental Safety*, 72: 1994-1998.

Talas, Z.S., Dundar, S.P., Gulhan, M.F., Orun, I. and Kakoolaki, S. 2012. Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic. *Iranian Journal of Fish Science,* 11: 405-414.

Wang, W., Li, T., Ning, Z., Wang, Y., Yang, B., Ma, Y. and Yang, X. 2012. A process for the synthesis of PUFA-enriched triglycerides from high-acid crude fish oil. *Journal of Food Engineering,* 109: 366-371.