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The Effects of Bee Bread (Perga) and Pollen on Liver Catalase Activity and Serum Antioxidant Levels in Quails

Arı Ekmeği (Perga) ve Polenin Bıldırcınların Karaciğer Dokusunda Katalaz Salınımı ve Serum Antioksidan Düzeyi Üzerine Etkisi

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ABSTRACT

This study was carried out to determine the effects of bee bread (perga, BB) and pollen on catalase immunoreactivity and serum antioxidant levels in liver tissue of quails. In the study, 30 1-day-old male Japanese quails (Coturnix coturnix japonica) were used. The quails were divided into three groups as control, bee bread and pollen groups. The animals were weighed on days 1, 14, and 28 of the study, and live weight values were determined for all groups. Body weight gain was calculated from the differences obtained from the weighings. At the end of the study, malondialdehyde (MDA) and glutathione (GSH) values were determined in the sera extracted from the blood samples taken at the end of the study, and liver tissues were taken and routine histological and immunohistochemical procedures were performed. Catalase immunoreactivity was evaluated in quail liver tissues. There was a statistically significant difference between the pollen group and the control group in terms of MDA levels. Strong catalase immunoreactivity was detected in the liver tissue of all groups. As a result, it is thought that the addition of pollen to the diet will contribute positively to the reduction of MDA levels.

Keywords: Bee bread, liver, pollen, quails

ÖZ

Bu çalışma, arı ekmeği (perga, BB) ve polenin bıldırcın karaciğer dokusunda katalaz immünoreaktivitesi ve serum antioksidan düzeyleri üzerindeki etkilerini belirlemek amacıyla gerçekleştirilmiştir. Çalışmada, 30 adet 1 günlük erkek Japon bıldırcını (Coturnix coturnix japonica) kullanılmıştır. Bıldırcınlar kontrol, arı ekmeği ve polen grupları olmak üzere üç gruba ayrılmıştır. Hayvanlar çalışmanın 1., 14. ve 28. günlerinde tartılmış ve tüm gruplar için canlı ağırlık değerleri belirlenmiştir. Vücut ağırlığı artışı, tartımlardan elde edilen farklardan hesaplandı. Çalışmanın sonunda, çalışmanın sonunda alınan kan örneklerinden ekstrakte edilen serumlarda malondialdehit (MDA) ve glutatyon (GSH) değerleri belirlendi ve karaciğer dokuları alındı ve rutin histolojik ve immünohistokimyasal prosedürler uygulandı. Bıldırcın karaciğer dokularında katalaz immünoreaktivitesi değerlendirildi. MDA düzeyleri açısından polen grubu ile kontrol grubu arasında istatistiksel olarak anlamlı bir fark vardı. Tüm grupların karaciğer dokusunda güçlü katalaz immünoreaktivitesi tespit edildi. Sonuç olarak, diyete polen ilavesinin MDA düzeylerinin azalmasına olumlu katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Arı ekmeği, bıldırcın, karaciğer, polen

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INTRODUCTION

Bee bread (BB) is, in fact, fermented and naturally preserved pollen. Pollen is collected by bees, mixed with their digestive enzymes, transported within the hive, and preserved under a thin layer of honey and beeswax. It is a fermented bee product that consists of pollen, honey, and bee saliva that passes through various chemical processes for 2 weeks facilitated by specific enzymes, microorganisms, humidity, and temperature (35-36°C). BB, which consists of a balanced set of proteins including all essential amino acids, vitamins (C, B1, B2, E, H, P, nicotinic acid, and folic acid), pantothenic acid, pigments, and biologically active compounds such as saccharase, amylase, phosphatases, flavonoids, carotenoids, and hormones, is a product with a high potential for use as a nutritional supplement. The emphasis on the chemical and therapeutic properties of BB has increased the interest in it worldwide.[1-5] Bee pollen is also used as a natural supplement because it contains most of the basic compounds needed for growth and development in the human body.[6,7] It contains essential amino acids, proteins, unsaturated fatty acids, anthocyanins, ferulic acid, pantothenic acid, vitamins, and minerals such as iron, manganese, and zinc. It also contains significant amounts of carotenoids, phytosterols, and polyphenolic substances like flavonoids. Polyphenols have a strong antioxidant capacity and the ability to scavenge free radicals.[8-10]

The livers of poultry and mammals are similar to each other in terms of their functional and structural properties. The liver has highly important functions against free radicals that have harmful effects on proteins, genes, and the entirety of cells. Antioxidants are substances that prevent the harmful effects of free radicals, and catalase is one of the main antioxidant enzymes. Catalase is found in almost all aerobic organisms. While various enzyme activities of catalase in the liver, kidneys, skeletal muscles, adipose tissue, and pancreatic islets have been reported, the highest levels of enzyme activity have been found in the liver.[11-14] The free oxygen radicals produced as a result of cellular metabolism lead to cell death. Antioxidants are compounds that inhibit or delay the initiation or acceleration of oxidizing chain reactions in the cells. The polyphenols found in BB and bee pollen reduce the formation of free oxygen radicals and metal ions. Previous studies have shown that BB also has antioxidant activities. In addition to these activities, BB is known to have protective effects on the human body, regulate metabolism, the liver, nervous system, and endocrine system, and rejuvenate tissues.[15] In this study, it was aimed to investigate the effects of BB and pollen on catalase secretion in the livers of quails and their serum antioxidant levels.

MATERIALS AND METHODS

For this study, thirty male, one-day-old Japanese quails chicks (Coturnix coturnix japonica) were obtained from the poultry unit at the Kafkas University Prof. Dr. Ali Riza Aksoy research and application farms. Approval was received for this research from Kafkas University Experimental Animals Ethics Committee (dated October 25, 2021, numbered 2021/10 and approval number KAU-HADYEK/2021-160). The quails were divided randomly into three groups as follows:

1. Control group (C) (n=10): Received no additives.
2. Bee bread group (BB) (n=10): Bee bread at a dose of 1g/kg added to diet for 35 days.
3. Pollen group (P) (n=10): Pollen at a dose of 1g/kg added to diet for 35 days.

During the study period, chicks were housed in species-specific cages (50 × 30 × 50 cm³). The quail chicks were fed with a diet (%22 HP and 3000 kcal/kg ME, Table 1) in the trial according to the NRC, (2001).[16] The feed and fresh water were provided as ad libitum to the animals. Ambient temperature was kept at 32-33 °C for the first three days and then gradually reduced by 1-2 °C each week and stabilized at 25 °C. With the exception first 3 d, the chicks were maintained under a 17-hour light/7-hour dark photoperiod throughout the experiment. The trial lasted for 35 days.

Table 1. Nutrient and chemical composition of quail diet.

Ingredients / Nutrients	%
Corn	56.56
Soybean Meal (44% CP)	39.50
Vegetable Oil	0.50
Limestone Powder	1.37
Salt	0.35
Dicalcium Phosphate (DCP)	0.80
Vitamin-Mineral Premix *	0.30
DL-Methionine	0.29
L-Lysine HCl	0.23
L-Threonine	0.15
Chemical Analyses	
Dry Matter (DM), %	89.81
Crude Protein (CP), %	22.00
Metabolizable Energy (kcal/kg)	30000.88
Calcium (Ca), %	0.82
Available Phosphorus (P), %	0.32

*: 8,800 IU of vitamin A, 2,200 IU of vitamin D3, 1 mg of vitamin E, 2.45 mg of vitamin K3, 8.8 mg of calcium D-pantothenate, 4.4 mg of niacin, 0.2 mg of folic acid, 6.6 mg of vitamin B12, 10 mg of biotin, 60 mg of manganese, 60 mg of iron, 5 mg of copper, 60 mg of zinc, 0.25 mg of cobalt, 1 mg of iodine, and 0.15 mg of selenium are all provided by the premix for each kilogram of diet.

The animals were weighed on days 1, 14, and 28 of the study, and live weight values were determined for all groups. Body weight gain was calculated from the differences obtained from the weighings. At the end of the trial, blood samples were collected from all groups prior to slaughter. Subsequently, the serum was stored at −20°C for later chemical analyses. All animals were slaughtered following ethical rules. Liver were removed form dressed carcasses.

Histological Procedure

The liver tissue samples were fixed in 10% formalin solution for 24 h. After routine histological processing, tissues were embedded in paraffin, 5 µm thickness serial sections were cut from paraffin blocks and Mallory's modified triple staining (Triple) was applied to the sections.

Immunohistochemical Procedure

The streptavidin-biotin-peroxidase technique, one of the indirect methods, was used to the sections taken to the lames coated the chrome aluminum gelatin. After deparaffinization and rehydration procedures, the sections were incubated for 10 minutes in 3% H_2O_2 prepared in 0.1 M PBS to prevent endogenous peroxidase activity. For reveal the antigens heat was applied at maximum temperature in the citrate buffer solution in a microwave oven for 10 min. Then incubated with Large Volume Ultra V Block solution (Thermo Scientific/ LOT: PHLT811) for 10 min. Catalase (Santa Cruz/ sc-271358) (1/500 dilution) primary antibody was added to the sections and kept for 1 hour in a humid environment at room temperature. The sections were washed with PBS and Biotinylated Goat Anti B Polyvalent (Thermo Scientific/ LOT: PHLT811) and Streptavidin Peroxidase solutions (Thermo Scientific/ LOT: PHLT811) were applied to the sections and incubated at room temperature for 15 min each. DAB-H_2 O_2 (Diaminobenzidine hydrogen peroxide) (Thermo Scientific/ LOT: HD53495) Substrate Solution was added for chromogen application and modified Gill III haematoxylin solution (Sigma-Aldrich/ LOT: HX29596474) was used for counterstaining. For the purpose of determining whether the immunoreactivities are specific to the quail liver sections taken from all groups, all procedures were kept in PBS without the addition of primary antibody (omission control) and the other procedures were applied the same. All sections were evaluated and photographed under the light microscope (Olympus BX51; Olympus Optical Co. Osaka, Japan).

Evaluations of the immunoreactivity were per formed semi-quantitatively as follows: no staining (0), weak staining (1), moderate staining (2), strong staining (3). Catalase immunoreactivity positive cells were counted made using image-j software program. Cell counting was performed by randomly selecting 6 slides from each group, from 4 areas on each slide, i.e. 24 areas in total, and compared between the groups.[17]

Biochemical Analyses

To asesse antioxidant state in serum extracted from blood samples, GSH levels were analysed following the procedure by Beutler et al.[18] and MDA levels were measured using the method described by Yoshoiko et al.[19]

Statistical Analyses

IBM Statistical Package for the Social Sciences version 20.0 (IBM PSSS Corp., Armonk, NY, USA) package program was used to evaluate the data obtained in the study. The difference between the results was analyzed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used for pairwise comparisons between groups.

RESULTS

Changes in Body Weight

The effects of supplementation of BB and pollen to quail diets on body weight and body weight gain are shown in Table 2. The body weight, body weight gain of the quails were not affected by the tested additives ($P > .05$).

Table 2. Effect of BB and polen on body weights and body weight gain.

Groups	N	1th day BW	14th day BW	28th day BW	Days 1-14th BWG	Days 15-28th BWG	Days 1-28th BWG
C	10	9.60±0.12	77.10±2.81	150.40±3.24	4.82±0.21	5.24±0.29	5.03±0.12
BB	10	9.59±0.11	84.10±3.02	153.00±3.79	5.32±0.22	4.92±0.32	5.12±0.14
P	10	9.58±0.16	85.40±4.28	158.00±3.30	5.42±0.30	5.19±0.31	5.30±0.12
P		0.997	0.203	0.302	0.205	0.745	0.300

C: Control; BB: Bee bread; P: Polen; BW: Body weight; BWG: Body weight gain.

Antioxidant Parameters

In the analyses of MDA, which is a product of the oxidant system, it was seen that the MDA levels in the BB and pollen groups were lower than those in the control group, whereas this difference was significant only between the pollen and control groups ($P < .05$). While the values of GSH, an indicator of the antioxidant system, increased in the BB and pollen groups in comparison to the control group, this difference was not statistically significant. The MDA and GSH levels of the groups are presented in Figure 1.

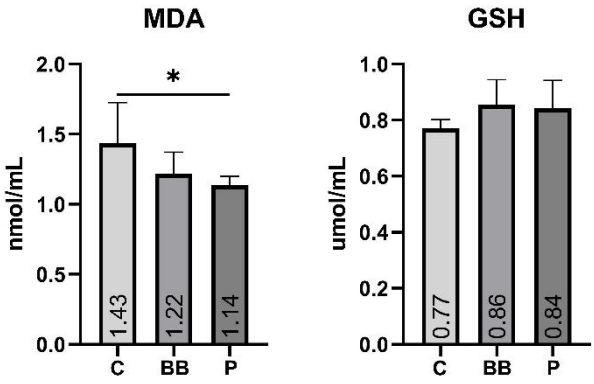

Figure 1. MDA and GSH levels of the groups. Control group: C, Bee bread group: BB, Pollen group: P. *: There is a significant difference only between pollen and control groups in terms of MDA levels ($P < .05$).

Histological Parameters

Vena centralis, hepatocytes, sinusoids, perisinusoidal spaces and lymphocyte infiltration were observed in the liver tissue of Japanese quails. The histological structure of Japanese quails liver tissue in all groups is given in Figure 2.

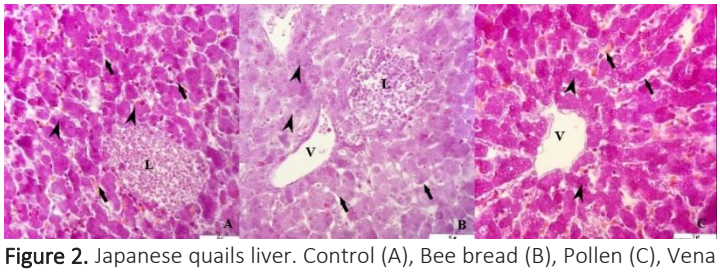

Figure 2. Japanese quails liver. Control (A), Bee bread (B), Pollen (C), Vena centralis (V), hepatocyte (arrowhead), sinusoid (arrow), lymphocyte infiltration (L). Triple staining. Bar:50µm.

Immunohistochemical Parameters

In the liver tissue of control, bee bread and pollen groups of Japanese quails, strong catalase immunoreactivity was determined in hepatocytes (Figure 3). In all groups, immunoreactivity was seen especially in the periphery of the tissue. The semiquantitative scoring results of Catalase immunoreactivity positive cells are shown in Table 3. No significant difference was observed between the groups in terms of score points. The mean score of group C was 2.86, group BB was 2.93 and group P was 2.88. Although the BB group had the highest average score, it was not statistically significant ($P < .05$).

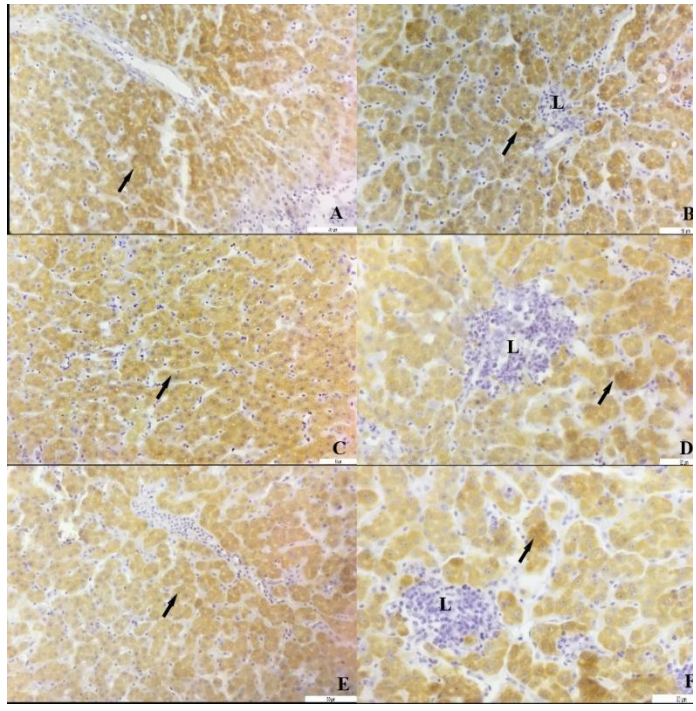

Figure 3. Catalase immunoreactivity in Japanese quails liver tissue. Control (A, B), Bee bread (C, D), pollen (E, F). Hepatocyte (arrow), lymphocyte infiltration (L). Bar:50µm.

Table 3. Semi-quantitative scoring results of catalase immunoreactivity positive cells.

Groups	N	Score
C	24	2.86±0.05[a]
BB	24	2.93±0.02[a]
P	24	2.88±0.05[a]
P		0.487

C: Control; BB: Bee bread; P: Polen. a: There is no difference between the averages shown with the same alphabet in the same column.

DISCUSSION

Pollen is the reproductive material of flowering plants produced in their male organs.[20] Honeybees store the pollen that they collect in the form of BB inside the cells of the hive. Pollen, honey, and other secretions of bees are mixed, and the mixture goes through lactic acid fermentation. In approximately two weeks, the mixture turns into BB, and this way, it can be stored for a long time in the hive. BB is a source of protein, fat, and vitamins for bees, as well as the main component of royal jelly. Although the compositions of bee pollen and BB are similar, they also have some differences. BB contains less protein compared to bee pollen, but it is easier to digest. Compared to bee pollen, BB contains 6 times more lactic acid, which facilitates its preservation. It was also reported that the taste characteristics of BB were better than those of bee pollen, and BB was easier to absorb in the body. [21]

The attack of reactive oxygen species on lipids, especially polyunsaturated fatty acids, is known as 'lipid peroxidation'. As a result of lipid peroxidation, aldehydes are formed. These aldehydes, which can be in several different biological forms, constitute bioactive molecules that create further oxidative damage. It was emphasized that MDA, which is one of the most frequently studied substances among these aldehydes, is effective in some pathological processes.[22] GSH is an essential antioxidant that is known to have significant roles in the detoxification of exogenous and endogenous substances such as xenobiotics, carcinogens, free radicals, and lipid peroxides.[23] It is one of the most important antioxidant substances that protect cells against the destructive effects of free oxygen species forming in the metabolism. It was reported that GSH levels were outside the normal limits in the pathogenesis of cancer.[24] In studies examining the effects of supplementing quail (Coturnix japonica) diets with clove oil (Syzygium aromaticum) and black seed oil (Nigella sativa) on growth performance and health parameters, it was seen that hepatic GSH, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S transferase (GST), and glutathione reductase (GR) levels increased, and MDA levels significantly declined in comparison to control groups. Moreover, increased feed intake and live weight were reported. It was proposed that using clove oil and black seed oil could increase the growth performance and antioxidant status of quails.[25]

In a study investigating the effects of bee products on feed performance and carcass characteristics, it was reported that products like propolis, pollen, and honey raised the growth performance of quails.[26] The addition of bee pollen into the rations of Japanese quails was seen to show a protective effect and regulate lipid peroxidation and the fatty acid composition of tissues.[27]

It was asserted that adding bee pollen into quail rations at different doses did not have a significant effect on their slaughter weight, carcass ratio, heart ratio, liver ratio, or gizzard ratio[28], while another study showed that it affected carcass and heart weights but did not affect gizzard and liver weights.[29] Changes in different biochemical parameters in the muscle and liver tissues of quails fed with two different doses of isoflavones for a prolonged period were analyzed, and it was reported that the levels of GSH, which is one of the most important criteria of the antioxidant capacity of living systems, showed an increase in the groups given isoflavones in both the liver and muscle tissues of the animals.[30] In our study, it was determined that daily increase in live weights, feed consumption, and feed conversion rate values were not significantly affected by BB or pollen supplementation, there was a significant decrease in MDA levels in the pollen group in comparison to the control group, and there was no significant difference among the groups in terms of their GSH levels.

CAT is an antioxidant enzyme that is localized in the mitochondria and peroxisomes and not in chloroplasts and neutralizes H_2O_2 by converting it into water and oxygen.[31] It was reported that an extract obtained from Ocimum basilicum leaves minimized the damage induced in the livers of BALB/c mice by paracetamol, reduced their MDA levels, and raised the immunoreactivity of SOD and CAT.[32] It was found that treatment with an extract obtained from Zingiber officinale and an active compound (6-gingerol) alleviated hepatorenal toxicity caused by mercuric chloride by reducing the MDA levels in rat livers and kidney and returning the activities of SOD, CAT, and GPx to normal values.[33] The effects of organic selenium (Se) on the expression of the CAT and GPx4 genes in broiler chickens (Gallus gallus) were analyzed, and it was reported that while liver CAT mRNA levels significantly decreased as the supplementation dose of Se increased in the 4th week, they were not affected by Se in the 6th week.[34] CAT and SOD immunoreactivity was found positive in all layers of the wall of the oviduct in chickens.[35] Treatment including poplar-type propolis was observed to cause healing in hepatic and renal lesions induced by streptozotocin, raised GPx levels, and lowered MDA levels.[36] In our study, strong CAT immunoreactivity was observed in the liver tissues of the quails in all groups. The finding in our study, MDA levels were found to decrease in line with the literature.[33,36] In addition, catalase immunoreactivity was determined in all groups, and no significant difference was observed between the groups.

In Japanese quails, there is a capsule with connective tissue consisting of collagen and elastic fibers surrounding the liver from the outside. Because the interlobular interstitium is much less available, the hepatic triad is in a scattered form. There are perisinusoidal spaces between hepatocytes and sinusoids. Erythrocytes around blood vessels and sinusoids and perivascular lymphocyte infiltrations around lymphatic vessels are encountered. Between the hepatic lobules, there are connective tissue zones known as the 'Kiernan space'.[37] The histological structures of the quail livers examined in this study were similar to those in the literature, and there was no significant difference among the groups.

As a result, treatment methods that originate from nature have been used from the past to the present because they have fewer side effects and are inexpensive. It is known that many conventional drugs produced today are obtained using medicinal plants, minerals, and organic substances. In

our study, we investigated the live weights, MDA and GSH levels, and liver CAT immunoreactivity statuses of quails fed by supplementing bee bread and pollen into their diets. Our results, there was a significant decrease in the MDA levels of the pollen group in comparison to the control group.

Ethics Committee Approval: Approval was received for this research from Kafkas University Experimental Animals Ethics Committee (Date: 25.10.2021, Number: 2021/10 and approval number: KAU-HADYEK/2021-160).

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