

MODULATING EFFECT OF PROPOLIS AND BEE POLLEN ON CHICKEN BREEDING PARAMETERS AND PATHOMORPHOLOGY OF LIVER AND KIDNEYS IN THE COURSE OF NATURAL INFECTION WITH *SALMONELLA* ENTERITIDIS

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Abstract

The liver and kidneys of broilers fed during the first two weeks of fattening a fodder containing the addition of 0.025% propolis and/or 0.5% pollen were examined macroscopically and microscopically. Additionally, the effects of these substances on the results of breeding and on pathomorphology of the liver and kidneys in the course of natural, asymptomatic infection with *Salmonella* Enteritidis were determined. The results demonstrated that propolis has protective effects on the liver of broiler chickens, reducing the intensity of regressive lesions. However, regarding supplementation of the diet with bee pollen, additional research on its effects in animals is needed.

Key words: broiler chicken, liver, kidney, propolis, bee pollen, *Salmonella* Enteritidis, pathomorphology.

Both propolis and bee pollen are valuable products to humans. To produce propolis, bees use substances gathered from leaf buds of various tree species or active compounds secreted by plants and released in areas of damage (e.g., lipophilic substances of leaves, plant glues and rubbers, resins). Propolis also contains bee saliva, together with the enzymes that compose it, and about 1,000 other various substances that have been chemically determined. Waxes make up a significant part of propolis (about 30%–40%), and the remainder is a mixture of various compounds such as volatile fatty acids, resins, balsams, and polyphenols (e.g., flavonoids, organic phenolic compounds, ketones, terpenes) (5, 13). Bee pollen, on the other hand, is made up of the male reproductive cells of plants. To date, about 250 various chemical compounds have been determined in it, including carbohydrates, fats, proteins, vitamins, macro- and microelements, antibiotics (inhibitors), hormones, enzymes, organic acids, phytoncides, essential oils, rutin, and others (21).

Because of this rich composition, both products exert a broad spectrum of positive effects on humans and animals, such as antibacterial, antifungal, antiviral, analgesic, and anti-inflammatory effects, as well as immunostimulating and immunomodulating influences

(2, 12, 14, 20). Furthermore, propolis and bee pollen reduce the risk of atherosclerosis (15) and high antioxidant activity of these products related to the flavonoid content, protect internal organs (liver, kidneys) against damage (mainly by toxic agents), stimulate regeneration of damaged tissues, and demonstrate an anti-carcinogenic effect (1, 3, 6, 7, 11, 16-18). Despite the positive influence of propolis and bee pollen on the health of animals, the information concerning the effect of these compounds on various parameters of farm animal breeding (consumption of fodder, body gains) and slaughter quality is quite scarce. Only a few studies have described the morphological pattern of internal organs after the application of these products in animals (2, 16).

Therefore, the aim of the experiment was to analyse the effect of adding propolis, bee pollen, or a mixture of propolis and bee pollen to fodder on breeding outcomes, slaughter quality, and morphology of the liver and kidneys in broiler chickens. Additionally, because of the occurrence of a natural infection with *Salmonella* Enteritidis, the effect of these products on the pathomorphology of the liver and kidneys in the course of salmonellosis of the latent type was also assessed.

Material and Methods

The research material consisted of 256 chickens of the Ross 308 breed, divided into four groups of 16 males and 16 females, in two independent experiments (a total of 16 groups of 16 animals in each). The breeding of birds lasted 6 weeks; during that time, the control broilers (group 1) received standard fodder while experimental birds in the first two weeks of fattening received standard fodder with the following additives:

- group 2 (propolis) – 0.25 kg of propolis/1 ton (250 mg/kg of fodder),
- group 3 (pollen) – bee pollen in the amount of 5 kg/1 ton (5 g/kg of fodder),
- group 4 (propolis and pollen) – bee pollen (5 kg/1 ton) and propolis (0.25 kg/1 ton).

Birds from all groups were kept under the same optimal zoohygienic conditions and under standard veterinary medical care.

Propolis used as an additive of fodder was subjected to a process of chemical standardisation to determine the amount of flavonoids as converted to galangin, a flavonoid significantly determining the biological activity and medicinal properties of propolis (9). The result of chemical standardisation was set at 2.5% of the quercetin content. Propolis in the form of dry extract (Phytopharm, Poland) was added to the feed in powder form. Then it was mixed thoroughly with the feed, which was administered in the loose form.

At the end of weeks 2, 5, and 6 of breeding, 12 chickens (6 cocks and 6 hens) were randomly selected from each group for slaughter and assessment of slaughter quality. Carcasses were subjected to detailed dissection, during which sections of the liver and kidneys were taken. This material was fixed in 10% buffered formalin. Afterwards, the tissues were dehydrated with increasing concentrations of ethyl alcohol (50%, 75%, and 98%), cleared in xylene, and embedded in paraffin blocks. Microtome sections (5 µm thick) were stained with haematoxylin and eosin and assessed under a light microscope (Nikon Eclipse E200).

The assessment of breeding indicators involved determination of the final body mass and share of individual elements (dorsal and chest parts, legs, neck, and wings) in a carcass. Additionally, the level of fodder consumption per 1 kg of weight gain was determined. The collected results were statistically analysed with the use of Statistica 8.0. The evaluation of slaughter quality was based on the analysis of the tissue composition of carcasses, *i.e.*, the ratios of muscle tissue to fat tissue and bones.

On the last day of the experiment, samples were collected for tests for the presence of *Salmonella* organisms according to the national anti-salmonellosis programme for poultry flocks. The results of laboratory examinations revealed the presence of *Salmonella* Enteritidis in the internal organs of all chickens.

Results

Slaughter quality and breeding results.

Neither the addition of propolis, nor bee pollen to fodder significantly affected body mass or the mass of chicken carcasses. The lowest body mass was found in birds from group 2. The weight shares of individual elements of carcass (dorsal and chest parts, legs, neck, wings) were similar in each group. The additives also had no significant effect on the use of fodder per 1 kg of weight gain. Regarding the tissue content, a significantly lower share of meat in relation to bones was found in carcasses of animals receiving propolis in comparison to the control birds (the following ratios were obtained: group 1 – 4.34, group 2 – 3.97, group 3 – 4.17, and group 4 – 4.03). On the other hand, the meat-to-fat ratio was similar in the broilers of all groups at about 4.0.

Microscopic pattern of the liver. In the liver, various regressive lesions were observed in hepatocytes, from slight parenchymal degeneration, through vacuolar degeneration and steatosis, to extensive necrosis (these lesions were noted in all birds during the dissection, when enlargement of the organ was observed, and they were accompanied by brittleness and paleness or marbled discolouration). The mildest and of the lowest intensity lesions, in the form of parenchymal and vacuolar degeneration, were observed in group 2. In the group of control birds, necrosis of single hepatocytes was found in one animal. On the other hand, the strongest retrogressive lesions were found in birds from groups 3 and 4, in which large areas of necrosis were observed, particularly in the vicinity of damaged bile ducts. Small hepatocytes with thickened cytoplasm (separation) were visible in birds from all groups, and no significant differences were found in the frequency or intensity of this lesion among the individual groups. Regarding regenerative-type lesions, binucleate hepatocytes were observed in single birds from groups 1 and 3. Additionally, single mitotic figures were present in hepatocytes in birds from all experimental groups.

Hyperplasia of bile ductular epithelial cells was frequently observed. In each group, some animals exhibited weak or moderate proliferation of biliary ductules (limited mainly to the areas of hepatic triad), and proliferating epithelial cells formed clear structures resembling proper biliary ductules (oval or round) (Fig. 1A). In other animals in each group, proliferation of biliary ductules was very strong. In this case, clusters of stimulated epithelial cells of biliary ductules were irregularly scattered within the parenchyma, where they formed non-organised streaks (Fig. 1B). Weak or moderate proliferation was visible mainly in birds from groups 1 and 4. It was stronger in group 3, and was very strong in half of the animals from the group receiving propolis.

No significant differences were found between the groups in terms of intensity or frequency of pathomorphological changes in bile ducts. In most chickens, various degrees of inflammation (cholangitis) were observed (Figs 1C, D). The most frequently observed lesion was the increase in the basophilia of epithelial cells. The epithelium was generally cubic, but

it was also cylindrical in one bird from group 4 and flat in one bird from group 2. Excessive proliferation was accompanied by exfoliation, leading to local losses. Necrosis or degradation of epithelial cells was often observed, while other regressive lesions included vacuolisation of the cytoplasm (one bird from group 3 and one from group 4). The mucous membrane was plicated, and shapeless masses and remains of disintegrated, exfoliated epithelial cells very often occurred in the lumen of the duct. The walls of the bile ducts were thickened through fibrosis and infiltrations of lymphocytes and mononuclear cells, and eosinophilic cells were occasionally found. A bile outflow was observed with heavy damage to the bile duct wall, which resulted in separation or necrosis of neighbouring hepatocytes.

The number and size of lymphatic follicles in the liver did not differ significantly among the groups. Rare, large single follicles were observed most frequently, as well as more numerous and small perivascular follicles. In one animal from group 3, lymphatic follicles revealed myeloid cells, while in one chicken from group 4, the follicle pattern was loose with the presence of connective tissue cells. Small and scarce clusters of myeloid cells were observed in three chickens from the group receiving bee pollen, in most birds from groups 1 and 2, and in all birds from group 4, in which myeloid cells additionally were abundant. Clear differences were observed in the case of lymphoid cells in liver parenchyma. They occurred most frequently in group 4, while in group 2, they were observed in only two birds. Lymphoid cells were visible most frequently near blood vessels while lymphocytes were scattered throughout the liver parenchyma.

Lesions that most frequently occurred in arteries in the liver included hypertrophy of wall tissue, increased acidophilia, stimulation of myocytes and vacuolisation of their cytoplasm (Figs 2A, B). Proliferation or swelling of endothelial cells and perivascular oedema occurred occasionally. Differences in character and intensification of lesions among groups were noticeable. In the control birds, they were found in half of the animals, while all chickens from group 4 displayed them, and the lesions consisted mainly of increased acidophilia and vacuolisation of myocytes. On the other hand, in the animals from the bee pollen group, lesions in the artery walls were found in four birds, but they were more intense and accompanied by a significant proliferation of myocytes and endothelial cells. Additionally, in one bird, these lesions also involved the veins, which showed thickening of the wall. In one case in group 4, inflammation of the veins with hyperplasia of connective tissue was observed with infiltration of inflammatory cells and adhesion of erythrocytes to the damaged wall.

Lesions frequently occurring in the liver included also widening of the lumen of the intralobular vessels, in which amorphous protein-like masses were visible. In addition, widening of the Disse space was observed. The groups did not differ significantly in the intensity of these lesions. Regarding other histopathological changes in the liver, the control birds

and chickens from group 4 revealed proliferation of connective tissue; in group 1, this proliferation was scattered throughout the parenchyma, while in group 4 it was located in the area of blood vessels. Slight fibrosis of the organ capsule was found in birds from group 2.

Taking into consideration the age of the birds, no significant differences were demonstrated in terms of the occurrence and intensification of lesions in hepatic tissue of the control group among birds at the age of 2, 5, and 6 weeks. The chickens from group 2 revealed a slight increase in damage to hepatocytes with age, while this group also demonstrated the weakest damage to the liver (most often only in the form of hepatocyte separation). On the other hand, in birds fed fodder with the addition of bee pollen or pollen and propolis, lesions in liver tissue were the strongest in the youngest animals, and weakening of their intensity was observed with age.

Microscopic pattern of the kidneys. In the macro- and microscopic patterns of the kidneys, no significant deviations from the proper structure of the organ were found. In the glomeruli, only a small widening of the lumen of glomerular capillaries and oedema of the visceral and wall epithelia were observed. Birds from groups 1, 2, and 3 did not differ in the intensity and type of the lesions, while lesions in group 4 were clearly less numerous and limited to widening of the lumen of capillaries. In the tubules, histopathological changes were occasional, and mainly concerned the distal tubules. In group 3, no deviation from their proper structure was recorded. In groups 1 and 4, however, widening of the lumen was observed in single animals, while the presence of a protein-like substance in the lumen was visible in one bird from group 1 and in one bird from group 2. Parenchymal degeneration of the epithelial cells of the tubules was found in one animal, also from the group receiving propolis. Slight kidney hyperaemia occurred in most chickens from group 1, in two birds each from groups 2 and 3, and in one bird from group 4. In the birds from all groups, inflammatory cell infiltrations were sporadically found, which in case of chickens from group 1 were located in the ureteral wall. An excessive growth of connective tissue was observed in two animals, one from group 2 and one from group 4.

Significant differences were found in the case of the so-called embryonic nephrons. Their number was clearly lower in birds from groups 3 and 4, while in the control group and the group receiving propolis, they covered large areas of the kidney parenchyma in half of the animals.

The number and size of lymphatic follicles did not differ significantly. Most frequently, they were scarce, small, and scattered throughout the parenchyma. Large and clear lymphatic follicles were visible in a few chickens from each group, and their number was small as well. Clusters of myeloid cells also occurred sporadically and were found only in group 2.

Microscopic lesions in the wall of the blood vessels were similar to those occurring in the liver. They involved the arteries and included hypertrophy of their walls, increased acidophilia and vacuolisation of

myocytes, proliferation or oedema of endothelium, and, in only a few chickens in each group, oedema of adventitia. No significant differences were found among individual groups in terms of the occurrence of these lesions and their intensity.

With reference to the age groups of chickens, lesions in kidneys revealed no significant differences.

Discussion

The results for breeding and slaughter indicators obtained in our experiment demonstrate that the addition of propolis caused a decrease in the body mass of birds and a reduction in the meat-to-bone ratio in elements of chicken carcasses. In the other experimental groups, the examined parameters did not significantly differ in comparison to the control birds. The results from the previous experiments assessing the effects of supplementation of the broiler diet with ethanol extract of propolis (EEP) suggested that it brings an improvement of breeding parameters such as body mass, growth, feed intake, feed conversion ratio, and

health parameters by, for example, reducing mortality rate (4, 10, 19). These findings seemed to suggest that the improvement in breeding parameters results from the antioxidant properties of flavonoids, which positively affect the condition of the alimentary canal (mainly through anti-microbial activity), digestion processes, and absorption of nutrients. Additionally, the high palatability of propolis components (waxes, resins, honey, and vanillin) was thought to increase fodder consumption, causing improvement in growth rates and increased body mass. The results obtained in our experiments in chickens infected with *Salmonella* Enteritidis did not reveal these positive effects of propolis, and for these parameters, the results were even worse than for birds from the control group, or the groups receiving bee pollen or bee pollen with propolis. This discordant result was probably caused by the very short period of propolis supplementation of fodder (only for the first two weeks); previous experiments demonstrated that higher weight increases were observed after 2–3 weeks of breeding with constant supplementation with high doses of EEP (10).

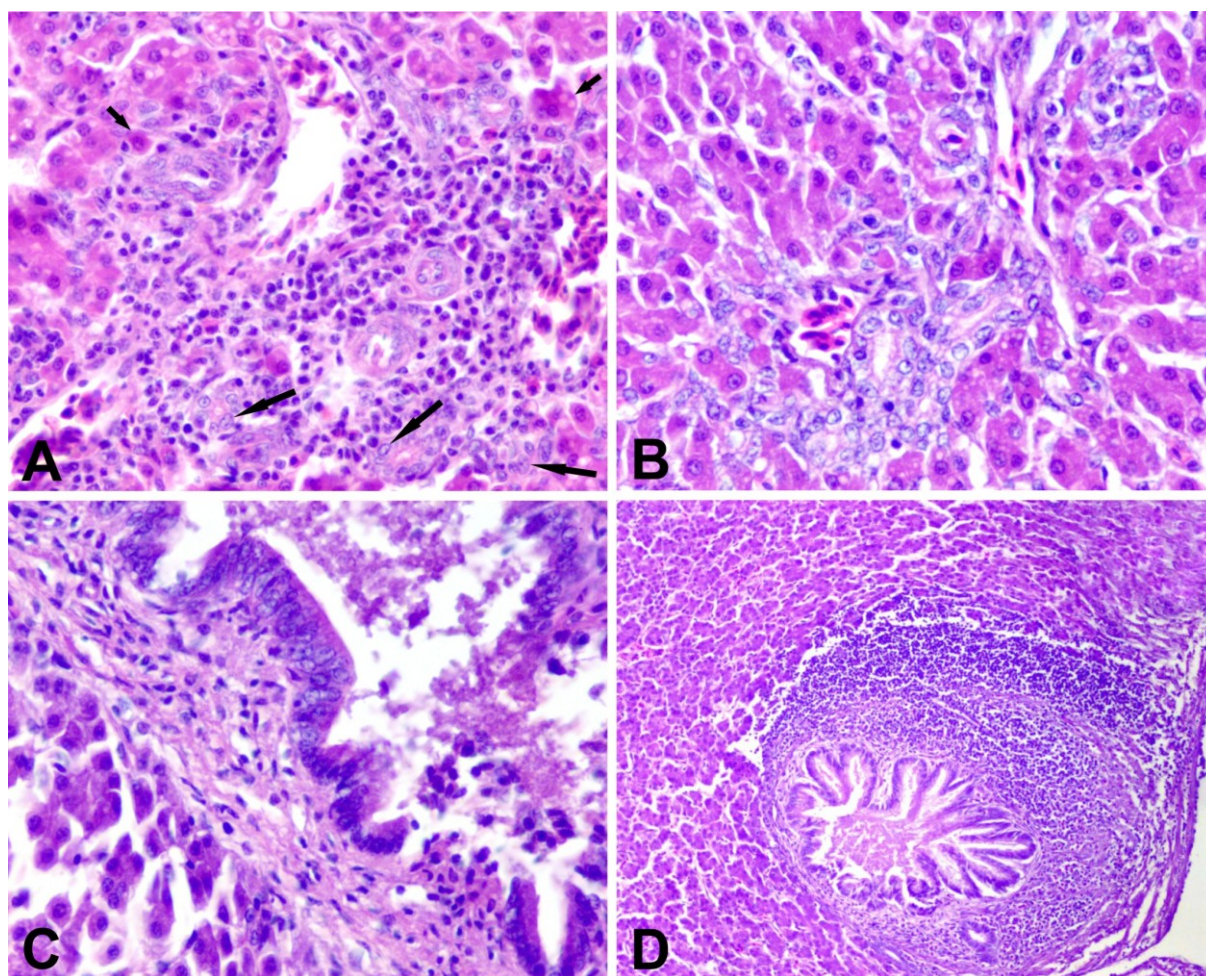


Fig. 1. Microscopic lesions in the liver tissue and biliary tract: **A** – Ductular proliferation (long arrows) with mononuclear cell infiltration, diffuse steatosis and necrosis of individual hepatocytes (short arrows), blood vessel injury (group 1, H.E., 400x); **B** – Hyperplasia of bile ductular epithelial cells and individualisation of hepatocytes (group 2, H.E., 400x); **C** – Bile duct damage surrounded by shrinkage hepatocytes (group 2, H.E., 400x); **D** – Severe cholangitis in bird from group 4 (H.E., 100x).

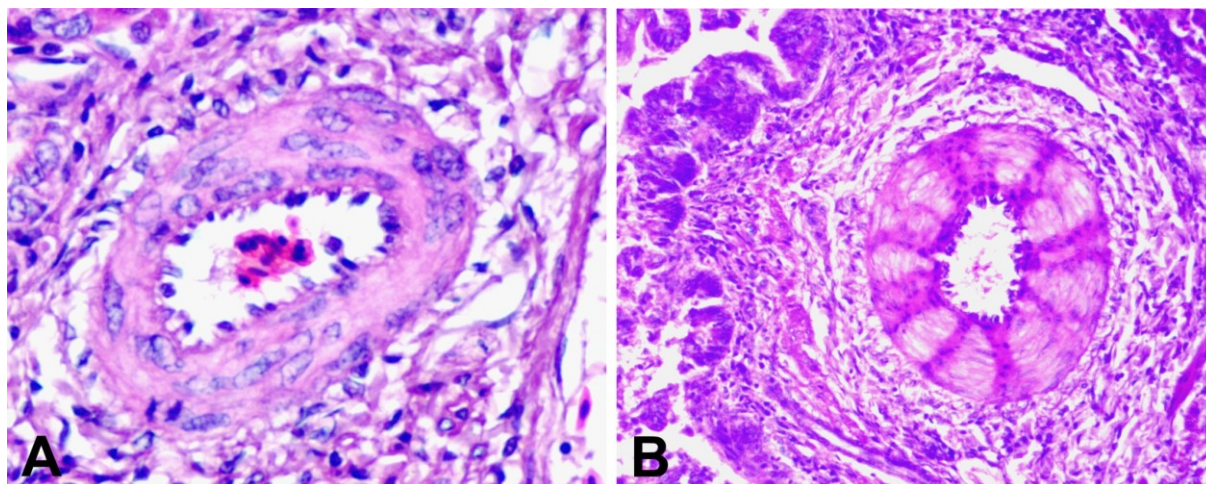


Fig. 2. Lesions in the wall of blood vessels: **A** – Significant stimulation of arterial myocytes and perivascular oedema (liver, group 3, H.E., 600x); **B** – Acidophilic myocytes in arterial wall, endothelial cells swelling, injury and proliferation of epithelial cells in bile duct (group 4, H.E., 400x).

The effect of bee pollen supplementation on breeding and slaughtering parameters of the examined birds remains an unresolved issue. The results obtained in our experiment can be interpreted in two ways, because there was no difference when compared with the control group, while in comparison with the group receiving propolis the obtained results were better in both: the pollen-only group and the combined supplement group.

This finding leads to two reasonable hypotheses: either pollen does not have a beneficial effect on the indicators assessed, or the effect of pollen is more beneficial in comparison to propolis. If we assume that bee pollen has a more positive effect on breeding parameters and slaughter quality of broilers, then on the basis of our results an assumption can be made claiming that propolis invalidates this beneficial effect. If so, a combined supplementation of bee pollen and propolis is not justified if the goal is better breeding and health effects.

The results of the limited research in the field show that the addition of propolis protects liver tissue against the negative effects of various hepatotoxic agents that lead to the occurrence of both regressive lesions, including necrosis, and progressive lesions, such as cancers. This property of propolis has been ascribed first of all to its phenol components (including flavonoids) and their antioxidant effect, which ensure protection against oxidation of fats in the content of cell membranes (16). Some research indicates that propolis also demonstrates an anti-cancer effect, not only within the liver tissue, but also in other organs (e.g., large intestine or against leukaemia) (1, 3). The results obtained in our research concerning the effect of propolis on liver morphology confirm its beneficial effect. The observed morphological lesions were the least intense in the broilers receiving only propolis supplementation. On the other hand, no significant differences were observed in birds fed fodder with the

addition of bee pollen or pollen and propolis in comparison to the control group. What is also significant is the fact that in those groups, contrary to the group receiving propolis, lesions decreased with the age of birds. This result indicates that propolis supplementation protects the liver against morphological lesions, which may be associated with more and more intense methods of fattening and poultry breeding, so it seems justifiable to supplement the diet with this product on a regular basis. Additionally, because of its properties, propolis can be a substitute for antibiotics and coccidiostats that have been withdrawn from common and prophylactic application in animal production.

The beneficial effects of propolis were also revealed in the case of blood vessels. In quickly growing broilers, pathological lesions develop rapidly in arteries, leading to thickening of their walls because of hyperplasia and/or hypertrophy of the smooth muscle or hyperplasia of connective tissue, which can result in narrowing or even closing of the lumen as a consequence of fibromuscular dysplasia (8). In our experiment the best results in this context were found for the group receiving propolis, which is consistent with the research carried out by Nadre *et al.* (15), who also found a protective effect of propolis on the condition of arterial vessels.

The current results are not a clear cut. Supplementation of fodder with propolis reduced the intensity of morphological lesions but slightly worsened slaughter indicators. On the other hand, opposite results were obtained in the case of pollen. The occurrence of a natural asymptomatic infection of chickens with *Salmonella* Enteritidis presents an additional difficulty in interpreting the results of our research. In the course of salmonellosis, damage to bile ducts and liver tissue can occur, as was observed during the dissection of the birds. Histopathological results demonstrated that in the group receiving propolis, damage to the organ caused by *Salmonella* Enteritidis infection was the weakest.

However, because of the natural character of the infection, it is difficult to precisely determine the extent of the protective effect of propolis on the liver tissue and bile ducts. Additionally, the finding of the most advanced lesions in chickens from the groups receiving pollen or pollen with propolis remains unexplained. Unfortunately, the available literature does not provide any clues regarding the reasons for such outcomes. Thus, further research on the effect of bee pollen in animals would be useful.

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