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Original article

The effect of propolis and bee pollen on the morphology of central lymphoid organs in broilers in course of natural infection with *Salmonella enteritidis*

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Abstract

Bee pollen and propolis are commonly used for therapeutic and prophylactic purposes in both humans and animals. This study evaluated their effects on the morphology of central lymphoid organs in broiler chickens. Birds were fed fodder supplemented with 0.025% propolis and/or 0.5% bee pollen during the first two weeks of fattening. Despite a natural, asymptomatic Salmonella enteritidis infection, no significant structural differences were observed in the thymus, spleen, or bursa of Fabricius. However, experimental groups – particularly those receiving propolis or bee pollen – showed signs of enhanced lymphoid activity and epithelial development. These findings suggest a protective and immunostimulatory effect of bee products on lymphoid organs, even during infection.

Keywords: bee pollen, bursa of Fabricius, chicken, histopathology, propolis, spleen, thymus

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Introduction

Propolis and bee pollen are biologically valuable natural substances that have been used in traditional medicine for centuries and are now subjects of growing interest in modern science due to their wide spectrum of biological and therapeutic properties (Pasupuleti et al. 2017, Żak et al. 2025). Propolis is a complex resinous mixture produced by bees from plant exudates, waxes, and enzymes, containing over 1,000 bioactive compounds such as flavonoids, phenolic acids, terpenes, and amino acids (Kasote et al. 2022). Its chemical composition varies depending on geographic origin, botanical sources, and season, making standardization of propolis-based products a challenge (Kasote et al. 2022).

The biological activity of propolis includes antimicrobial, antiviral, antifungal, anti-inflammatory, antioxidant, and immunomodulatory effects (Pasupuleti et al. 2017, Zulhendri et al. 2021). It has been shown to inhibit a wide range of pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, and *Helicobacter pylori*, and demonstrates antiviral activity against viruses such as HSV-1 and SARS-CoV-2 (Yosri et al. 2021). The mechanisms of these effects involve modulation of inflammatory signaling pathways like NF-κB and MAPK, inhibition of COX-2, as well as stimulation of phagocytic cell activity, nitric oxide (NO) production, and T-cell proliferation (Pasupuleti et al. 2017).

Bee pollen, another bee-derived product, is a nutritional complex composed of flower pollen, nectar, and bee gland secretions. It is rich in protein (20-30%), essential amino acids, vitamins (especially B, C, D, and E), trace minerals, and polyphenolic compounds such as quercetin and kaempferol (El Ghouizi et al. 2023, Anjum et al. 2024). Bee pollen has been documented to possess antioxidant, immunostimulatory, antibacterial, antifungal, hepatoprotective, and cardioprotective activities. It influences both cellular and humoral immune responses and its biological potential is further enhanced by novel technologies such as microencapsulation and nanoparticle delivery systems to improve bioavailability (Anjum et al. 2024).

Both bee products are increasingly considered promising nutraceuticals in animal and human nutrition, especially in the context of rising antibiotic resistance and the need for natural immunomodulators (Kieliszek et al. 2023). Although many studies have explored their systemic biological effects, limited data exist regarding their direct influence on the morphology and development of immune system organs in animals.

Numerous publications discuss the effects of propolis and bee pollen on immune function, but few investigate their impact on lymphoid tissue architecture. Therefore, the aim of this study was to evaluate the effect of dietary supplementation with propolis, bee pollen, or their combination on the morphology of primary lymphoid organs (thymus, spleen, and bursa of Fabricius) in broiler chickens exposed to a latent infection with *Salmonella enteritidis*.

Materials 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 I) received standard fodder and experimental chickens in the first two weeks of fattening received standard fodder with the following additives:

- group II (propolis) – 0.25 kg propolis/1 ton (250 mg/kg of fodder),
- group III (bee pollen) – 5 kg/1 ton (5 g/kg of fodder),
- group IV (propolis and pollen) – bee pollen 5 kg/1 ton and propolis 0.25 kg/1 ton of fodder.

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

Propolis used as an additive of fodder was subjected to a process of chemical standardization to determine the amount of flavonoids as converted to galangin, a flavonoid significantly determining the biological activity and medical properties of propolis (Fan et al. 2014). The result of chemical standardization 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. Dry bee pollen was supplied from an apiary of the Department of Apiculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Poland. Propolis and bee pollen, in powder form, were mixed with dry feed (Kleczek et al. 2012).

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. Carcasses were subjected to detailed dissection, during which samples of the bursa of Fabricius, spleen and thymus were taken. This material was placed in 10% buffered formalin. Afterwards, the tissues were dehydrated with increasing concentration of ethyl alcohol (50%, 75% and 98%), clear in xylene and embedded in paraffin blocks. Microtome sections 5 μm thick were stained with hematoxylin and eosin and assessed under light microscope (Nikon Eclipse 80i).

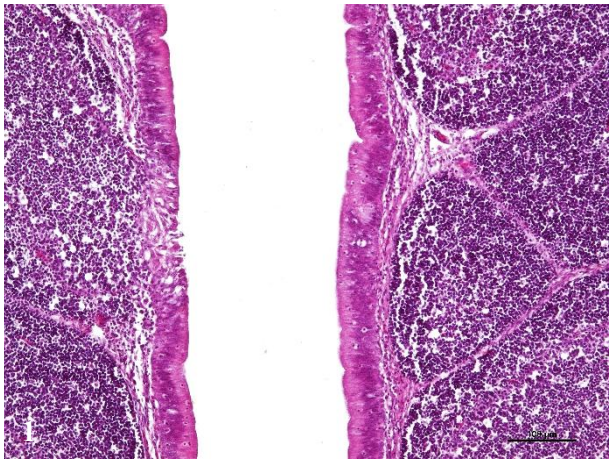

Fig. 1. Numerous intraepithelial lymphocytes and long segment of specilized epithelial cells. Bursa of Fabricius, chicken, 5-week-old, group II. HE staining. Scale bar = 100 µm.

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

No procedures involving pain or distress to animals were performed, and histological material was collected post-mortem; hence, approval from the animal ethics committee was not necessary.

Results

Macroscopic observations

The macroscopic appearance of the bursa of Fabricius, spleen, and thymus did not differ significantly among groups at any age. All organs were well-developed and showed no visible abnormalities.

Microscopic observations

Bursa of Fabricius

At 2 weeks of age, the overall bursal architecture was comparable in all groups. The luminal epithelium was mostly cuboidal to slightly folded. In the control group, it was composed of cylindrical cells, while in the combined supplemented group (IV) it was flatter. Broilers receiving propolis showed epithelial activation with locally cuboidal or columnar cells and intraepithelial lymphocytes; eosinophils occurred sporadically.

Follicle-associated epithelium (FAE) was least developed in groups I and IV, consisting of low cells with weak pinocytosis, whereas in groups II and III (propolis and pollen, respectively) large areas of activated FAE with numerous lymphocytes were visible. Follicles were numerous and cell-rich in all groups, with denser cortices than medullae. The control group showed fewer lymphoid cells in the medulla and rare mitotic figures; single apoptotic bodies appeared in groups I and III. One control individual exhibited extensive follicular necrosis with haemorrhages and eosinophilic infiltration.

With age, the bursa developed normally in all groups, showing higher folds, more follicles, and denser lymphoid parenchyma. In 5–6-week-old birds, epithelial folding and intraepithelial lymphocytes were most pronounced in propolis- and pollen-supplemented groups (Fig. 1). In group IV, the luminal epithelium was damaged above oedematous areas, and mild epithelial swelling was present. In one bird from group III, small perivascular mononuclear infiltrates appeared. Vascular myocyte vacuolization was seen in groups II and III, while small haemorrhages occurred sporadically in all groups, especially group IV (Fig. 2).

Spleen

At 2 weeks, spleen structure was typical in all groups. The capsule and subcapsular muscle layer were thin, except for slight thickening in the pollen group. In the control group, numerous mitotic figures were visible in the white pulp, while other groups showed fewer mitoses and apoptotic cells.

Periarterial lymphoid sheaths (PALS) contained immature lymphoid cells and small aggregates of dendritic cells and macrophages. Poorly defined B-cell follicles were present, and ellipsoids around penicillar arteries were small.

By week 5, PALS enlargement and lymphoid proliferation were evident in all birds, most prominently in propolis and pollen groups. These groups showed higher numbers of mature lymphocytes, especially in central PALS and follicles. In contrast, the combined group (IV) contained mainly immature lymphoblasts and cen-

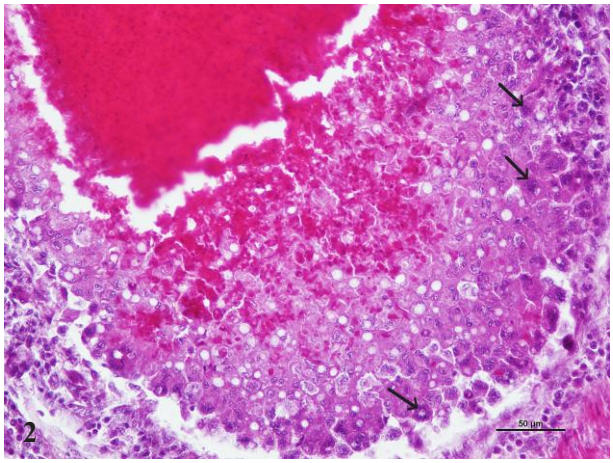

Fig. 2. Numerous apoptotic bodies (arrows) in a bursa-dependent lymphoid follicle. Spleen, chicken, 6-week-old, group III. He staining. Scale bar = 50 µm.

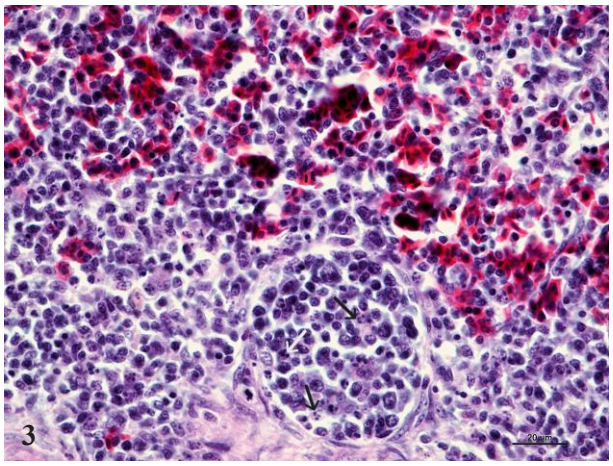

Fig. 3. Bursal abscess with syncytial cells formation (arrows). Bursa of Fabricius, chicken, 6-week-old, group IV. HE staining. Scale bar = 20 µm.

troblasts. Ellipsoids were numerous and composed of stromal and dendritic cells. Apoptotic bodies were frequent in the white pulp of groups I–III (Fig. 3) but sparse in controls; single mitotic figures occurred in the propolis group. Minor vascular thickening and perivascular oedema were noted sporadically, particularly in group III.

At 6 weeks, spleens were well-organized, with numerous small ellipsoids and thickened arterial walls composed of vacuolated or eosinophilic myocytes. These vascular changes were most pronounced in control birds. Occasional haemorrhages occurred in some 5-week-old controls.

Thymus

At 2 weeks, cortical and medullary architecture was preserved in all groups. Lymphoblasts predominated in the cortex of groups I–III, while mature lymphocytes dominated in group IV. The medulla contained reticular and epithelial cells, plasmocytes, and macrophages. Macrophages were particularly numerous in the propolis group. Vacuoles were rare, except for group II, where they were frequent and clustered. Single Hassall's corpuscles appeared only in the pollen group. Apoptotic bodies were scarce, with slightly higher numbers in group III.

At 5 weeks, all thymuses contained abundant lymphoid cells in both cortex and medulla. Mature lymphocytes dominated in groups receiving propolis or pollen. The medulla showed a mixture of lymphoid, reticular, and myeloid cells, with eosinophils more frequent in group III. Hassall's corpuscles and vacuoles did not differ notably among groups, and mitotic and apoptotic activity remained low.

By week 6, the thymic cortex contained dense lymphocyte populations, particularly at the periphery, while the medulla showed variable numbers of immature cells. In propolis- and pollen-supplemented groups, centrally located lobes contained more mature lymphocytes than peripheral ones. Eosinophilic cells were scattered in most birds, but more numerous in some from

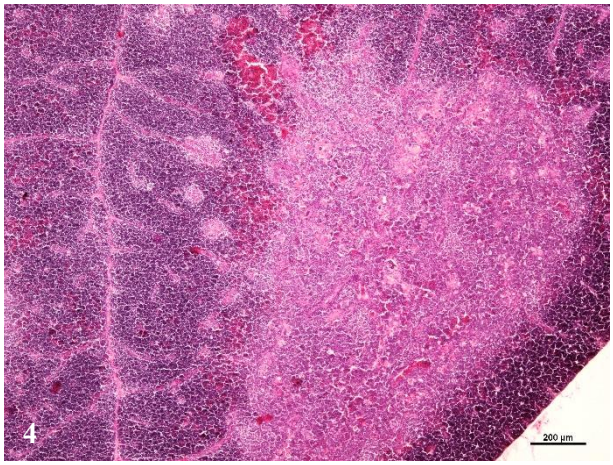

Fig. 4. Extensive extravasations in the thymus parenchyma. Thymus, chicken, 2-week-old, control group. HE staining. Scale bar = 200 µm.

group II. Vacuoles were small and few in all groups; only occasional individuals in groups II and IV showed a slight increase. Typical concentric Hassall's corpuscles were seen mainly in propolis-fed birds, while the control group had the highest overall number. Apoptosis and mitoses were rare in all groups.

Mild to moderate thymic oedema was found in some birds from groups I and III, and more pronounced in all group III individuals. Single cysts lined with ciliated epithelium and filled with amorphous material were present in several birds from group III. Focal haemorrhagic cysts and siderocytes occurred sporadically in groups II and IV, and one control bird showed large extravasations with mononuclear infiltration (Fig. 4).

Discussion

The search for natural alternatives to antibiotic growth promoters has intensified in recent years, directing attention to bee-derived products such as propolis and bee pollen. Both exhibit antimicrobial, antioxidant, and immunomodulatory properties (Pasupuleti et al. 2017, Zulhendri et al. 2021, Żak et al. 2025). However, information concerning their direct effect on the microscopic structure of avian immune organs remains scarce. Previous studies focused mainly on organ weights as indicators of immune stimulation (Sforcin 2007, Fan et al. 2013, Fan et al. 2014, Daneshmand et al. 2015). The present study provides a histological evaluation of the thymus, spleen, and bursa of Fabricius in broilers supplemented with propolis and/or bee pollen under conditions of natural Salmonella enteritidis infection.

No macroscopic abnormalities were found in any group, confirming the safety of both additives. Histologically, propolis and bee pollen induced mild but consistent stimulation of lymphoid and epithelial compartments in the examined organs. These effects were most evident in birds receiving single additives and less pronounced in the combined supplemented group, which largely resembled controls.

In the bursa of Fabricius, the most distinct response to propolis and bee pollen was activation of the follicle-associated epithelium (FAE) with cuboidal to columnar cells and numerous intraepithelial lymphocytes (Fig. 1). This indicates enhanced antigen sampling and B-cell activation, consistent with the concept that antigenic contact drives post-hatching maturation of B lymphocytes (Vainio and Imhof 1995, Ratcliffe 2006). Despite comparable numbers of apoptotic bodies across groups, the denser lymphoid parenchyma in supplemented birds suggests enhanced proliferation or prolonged survival of lymphocytes. The increased folding of the luminal epithelium and higher follicular density support this interpretation.

In the spleen, propolis and bee pollen promoted lymphoid proliferation within periarterial lymphoid sheaths (PALS) and follicles (Fig. 3). The presence of numerous apoptotic figures, together with a normal lymphoid density, indicates a high turnover of immune cells typical of physiological activation rather than degeneration (Yang et al. 2010, Cui et al. 2011, Li et al. 2015). Thickened arterial walls and minor perivascular oedema, especially in control birds, likely reflect vascular adaptation to increased functional demand. The well-preserved architecture of the spleen supports the view that both additives enhance immune organ activity without adverse structural effects.

In the thymus, supplementation slightly increased the proportion of mature lymphocytes in the cortex and medulla, and Hassall's corpuscles were more frequent in birds receiving propolis (Fig. 4). These structures are associated with thymic epithelial cell activity and negative selection of autoreactive T lymphocytes (Boes and Durham 2016). Therefore, the observed pattern may indicate a beneficial modulation of central tolerance. The limited apoptotic activity observed is consistent

with physiological thymocyte selection and confirms the absence of cytotoxic or degenerative effects. Cystic and oedematous changes recorded sporadically, particularly in the pollen group, likely represent mild physiological variability.

An important observation was the lack of synergistic effect when propolis and bee pollen were administered together. The combined supplementation did not enhance morphological stimulation beyond that observed for single additives. This may result from overlapping active compounds or reduced bioavailability when used simultaneously, as suggested in other studies on natural mixtures (Orsi et al. 2005, Daneshmand et al. 2015).

The natural, asymptomatic Salmonella enteritidis infection present during the experiment complicates interpretation. Mild vascular alterations and sporadic haemorrhages observed in some birds most likely reflected the background infection rather than the effect of dietary additives. Importantly, the severity of these lesions appeared reduced in the supplemented groups compared with controls, suggesting that propolis and bee pollen alleviated infection-related vascular reactions and supported the maintenance of immune organ integrity. The preserved morphology and active lymphoid structures in experimental groups indicate a stabilizing and potentially protective influence of bee products on the immune system. This finding is consistent with previous reports describing their antibacterial and anti-inflammatory properties (Medeiros et al. 2008, Chirumbolo 2011).

In summary, the present study demonstrates that propolis and bee pollen, administered individually, enhance the histological development and functional activity of the bursa of Fabricius, spleen, and thymus in broilers. These effects are manifested by intensified lymphoid proliferation and epithelial activation without evidence of tissue damage. The results highlight the value of microscopic assessment in evaluating immune stimulation, as organ weight alone does not reflect the true cellular differentiation or immune competence. Further studies employing immunohistochemical markers (e.g., PCNA, TUNEL) in healthy flocks are recommended to confirm these findings under controlled conditions.

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