Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Effect of Feed Supplementation with Propolis on Liver and Kidney Morphology in Broiler Chickens

I. Babińska*, K. Kleczek[1], W. Makowski[1] and J. Szarek

Department of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 13, 10-719 Olsztyn; [1]Department of Commodity Science and Animal Improvement, Faculty of Animal Bioengineering, ul. Oczapowskiego 5, 10-719 Olsztyn, Poland
*Corresponding Author: izabella.babinska@uwm.edu.pl

ARTICLE HISTORY

Received: December 23, 2011
Revised: March 11, 2012
Accepted: July 12, 2012

Key words:
Broiler
Histopathology
Kidney
Liver
Propolis

ABSTRACT

Propolis is a resin-like mixture produced by bees and composed of natural substances demonstrating a broad range of biological activity, e.g. antibacterial, anti-inflammatory, anti-oxidizing, immunostimulating and immunomodulating effects. Among abundant research on its properties has been carried out but a few reports concerning the use of this substance in animal production, particularly in poultry breeding are available. There is little information about its effects upon the morphology of individual organs and tissues predisposed to damage as a result of intensive fattening of poultry. A-day old 400 chicks were divided into equal four groups. Group 1 and 2 served as negative and positive control, respectively. Group 3 and 4 were kept on propolis (10 and 50 mg/kg of feed) supplemented feed for 42 days, respectively. At the end, randomly selected 12 birds from each group were killed humanly. Gross lesions were noted and liver and kidney samples were processed for histopathological studies. The results demonstrated a protective effect of propolis particularly upon the liver of broiler chickens, in which it reduced the intensity of regressive lesions. This protective effect was noticeable especially in the group of birds receiving a higher dose of propolis.

To Cite This Article: Babińska I, K Kleczek, W Makowski and J Szarek, 2013. Effect of feed supplementation with propolis on liver and kidney morphology in broiler chickens. Pak Vet J, 33(1): 1-4.

INTRODUCTION

Propolis, also referred to as bee glue, is a brownish resin-like substance gathered by bees from the leaf buds of various species of trees. It is also a component of substances actively secreted by plants or damaged parts of plants (lipophilic substances of leaves, plant glues and rubbers, resins, etc.). Propolis is also rich in bee saliva and enzymes (Marcucci, 1995). The natural therapeutic properties of propolis have been known since ancient times of Aristotle. Propolis has been known for its antibacterial, antifungal, antiviral and antiparasitic properties (Marcucci, 1995; Bevilacqua *et al.*, 1997; Banscota *et al*., 2001; Kimset *et al*., 2008). It has displayed anti-inflammatory, immunostimulating and immunomodulating effects (Banscota *et al*., 2001; Castaldo and Capasso, 2002; Sforcin, 2007; Khorasgani *et al*., 2010). In addition, it was found to have a protective effect on liver damage, mainly through its anti-oxidizing properties (Banscota *et al*., 2000; Bhadauria and Nirala, 2009; Nirala and Bhadauria, 2008) and it possessed the ability to stimulate tissue regeneration. In humans, it was reported to reduce the blood pressure, the level of LDH cholesterol and fat in blood which, in turn, reduced the risk of atherosclerosis (Nader *et al*., 2010). It evoked an analgesic effect comparable to that produced by cocaine (Sforcin, 2007). The research carried out in recent years has focused on its anti-cancer activity and revealed that its chemopreventive and cytotoxic properties against cancer cells are mainly because of a substance known as caffeic acid phenethyl ester-CAPE (Bazo *et al*., 2002; Aso *et al*., 2004; Borbath *et al*., 2007; Said *et al*., 2010).

Chemically, propolis contains about 1,000 components, 400 of which have been studied so far. These are mainly polyphenols, which include flavonoids, esters, phenolic aldehydes, ketones, terpenes and others. Additionally, propolis is composed of, among others, volatile fatty and aromatic acids, waxes, resins, balsams, and flower pollen (the source of such macroelements as magnesium, nickel, calcium, iron and zinc). The chemical composition of propolis depends on the local plant population, climatic conditions, periods in which

substances used for its production are gathered, as well as on the species of bees (Bevilacqua *et al*., 1997). In addition, the methods of obtaining extracts from propolis (water, alcohol, oil) influence its properties.

The abundant research on the beneficial properties of propolis has shown its positive effect on both human and animal health. On the other hand, the available literature does not provide sufficient data concerning the effects of its application on farm animals, particularly poultry. Information concerning the effect of propolis on tissues and organs of birds is also rather scarce (Khojasteh Shalmany and Shivazad, 2006; Kleczek *et al*., 2007a, b; Seven *et al*., 2008). Consequently, this research was undertaken to analyse the influence of the feed supplementation with propolis on the morphological picture of liver and kidneys of broiler chickens.

MATERIALS AND METHODS

The research material consisted of 400 chicks of the Ross 308 genetic line, which were divided into four groups of males and females (n = 25), in two replications (16 groups in total): group 1 (control negative) – base feed, group 2 (control positive) – base feed supplemented with 10 mg/kg flavomycin (growth stimulator) and 500 mg/kg robenidine (coccidiostat), group 3 – base feed supplemented with standardized propolis in the amount of 10 mg/kg of feed, group 4 – base feed supplemented with standardized propolis in the amount of 50 mg/kg of feed.

Feed mixtures were purchased in the "Morawski" Feed Production Plant, Kcynia, Kujawsko-Pomorskie Province. The raw material composition included wheat, wheat bran, soya meal, feed chalk, salt, vitamin and mineral additives. Birds were reared for 6 weeks. Chickens from all groups were reared according to the recommended technology and subjected to the standard prophylactic programme established and conducted by the veterinary surgeon.

Propolis used as feed supplement underwent a chemical standardization process to determine the amount of flavonoids converted to galangin, i.e. the flavonoid significantly determining biological activity and therapeutic properties of propolis (Kędzia and Hołderna-Kędzia, 1996).

At the end of rearing (42 days), 12 birds (6 roosters and 6 hens) from each group were selected at random and humanly killed for necropsy examination. Liver and kidney samples were taken from the birds directly after slaughtering and fixed in 10% neutralised formalin. The tissues were then dehydrated with increasing concentrations of ethyl alcohol (50, 75, 98 and 100%), cleared in xylene and embedded in paraffin. 5 μm thick microtome sections were stained with hematoxylin and eosin and assessed under a light microscope (Nikon Eclipse E-200).

RESULTS

The production performance and meat quality results of this study have been published elsewhere (Kleczek *et al*., 2007a, b).

Liver: The macroscopic examination did not reveal any significant differences in the appearance of the liver in individual groups of animals. The liver was properly developed in all birds. In group 1, fine, numerous cream-coloured spots were observed in parenchyma and, in one animal, the organ capsule was slightly thickened, turbid and opaque. On the other hand, in groups 2 and 3, in most chickens the liver was of brittle consistency, slightly enlarged and had rounded edges.

The liver of chickens from group 1 (control negative) showed small foci of necrosis and individualization of hepatocytes. A strong hyperplasia of *bile ductule epithelium* (bile ductule hyperplasia) was observed. In the area of the hepatic triad, *bile ductular epithelial cells* formed round or oval structures resembling normal ductules (typical bile ductule hyperplasia – Fig. 1A). On the other hand, clusters scattered in parenchyma were irregular and irregularly distributed, with slightly noticeable lumen (oval cell proliferation – Fig. 1B). Scarce small lymphatic follicles could be observed, located in close vicinity of blood vessels. Additionally, many aggregates of lymphoid cells and clusters of myeloid cells were found. In one bird, the capsule of the liver showed a low degree of fibrosis.

The liver in group 2 (control positive) showed a slight degree of vacuolar degeneration of parenchymatous cells. Some areas showing swelling of hepatocytes were also visible. Hepatocyte nuclei were from small ones of dense chromatin, to large and light with chromatin threads and nucleolus. Bile ductule hyperplasia was present both in the area of triads and in the parenchyma, where clear stripes of bile ductular epithelial cells were observed. Scarce, but clear and large lymphatic follicles were also noticed. Clusters of myeloid cells were single and small; their perivascular infiltration was very extensive only in one case (Fig. 1C).

The liver of birds from group 3 demonstrated slight focal vacuolar degeneration. Some hepatic cells have large, active nuclei. Lymphatic follicles scattered in parenchyma were also occasionally observed. Infiltrations of eosinophilic cells were clear and vast. Bile ductule hyperplasia was similar to the control negative group.

No regressive lesions were found in the liver of birds from the group receiving the feed supplemented with propolis in the amount of 50 mg/kg of feed. The nuclei were varied, just as in group 2. Lymphatic follicles were quite numerous and clear and some of them were very large. Small infiltrations of eosinophilic cells were observed near blood vessels. Hyperplasia of bile ductule epithelium was much weaker than in all other groups and it occurred mainly in the area of hepatic triads, while it was stronger near large blood vessels.

No significant differences were found between groups as regards bile ducts. The cuboidal epithelium having relatively basophilic cytoplasm lined the bile ducts. Group 3 revealed inflammatory lesions in the form of infiltration of mononuclear cells and lymphocytes in the bile duct wall, as well as epithelial defects. In all birds, bile ducts were patent, and bile with a small amount of cell aggregate (flaked epithelial cells) was often visible in their lumen.

Lesions in blood vessels were prominent in the positive control group. Artery walls demonstrated

significant induration and single myocytes of increased acidophilia and vacuolated cytoplasm were visible. Swelling and hyperplasia of endothelial cells was sporadically observed. A fibromuscular dysplasia of the inner membrane was also observed (Fig. 1D). In individual animals from this group, swelling of the cells of tunica adventitia was also found. Experimental groups demonstrated only slight induration of artery walls, whereas it was not related to changes in myocytes. Additionally, in group 1, low-degree fibrosis of venous vessels was found in one bird only.

Kidneys: During necropsy, no irregularities in the macroscopic appearance of kidneys were observed. In all birds, the organ was brown, properly developed and had an elastic consistency.

The microscopic pattern of kidneys revealed no significant aberrations from the proper structure of the organ. In glomeruli, a small widening of the lumen of blood vessels and swelling of the Bowman's capsule was often observed. No differences in the intensity or type of lesions in kidneys of chickens from groups 1, 2 and 3 were found, whereas in group 4, the microscopic pattern showed widening of the lumen of glomerular capillaries only. Histopathological lesions in the tubules were also rare and scarcely encountered in distal tubules. In birds from groups 3 and 4, no deviations from their normal structure were found, while in groups 1 and 2, widening of the lumen of the tubules was observed in some individual birds. Parenchymal degeneration of epithelial cells of the tubules was found in one animal from the group receiving propolis in the amount of 10 mg/kg of feed. Low-intensity plethora of kidneys occurred in most chickens from both control groups and in individual birds from the experimental groups. In all examined birds, the occurrence of inflammatory cell infiltration was sporadically present. Significant differences were observed in the so-called "embryonic" nephrons. Their numbers were clearly lower in the experimental groups 3 and 4, while in both control groups, they covered large areas of the kidney parenchyma in half of the birds examined.

No significant differences in the number or size of the interstitial lymphoid follicles were found. They were mostly scarce, small, dispersed and poorly expressed. Large and clear lymphoid follicles were visible only in single chickens from each group and their number was also low.

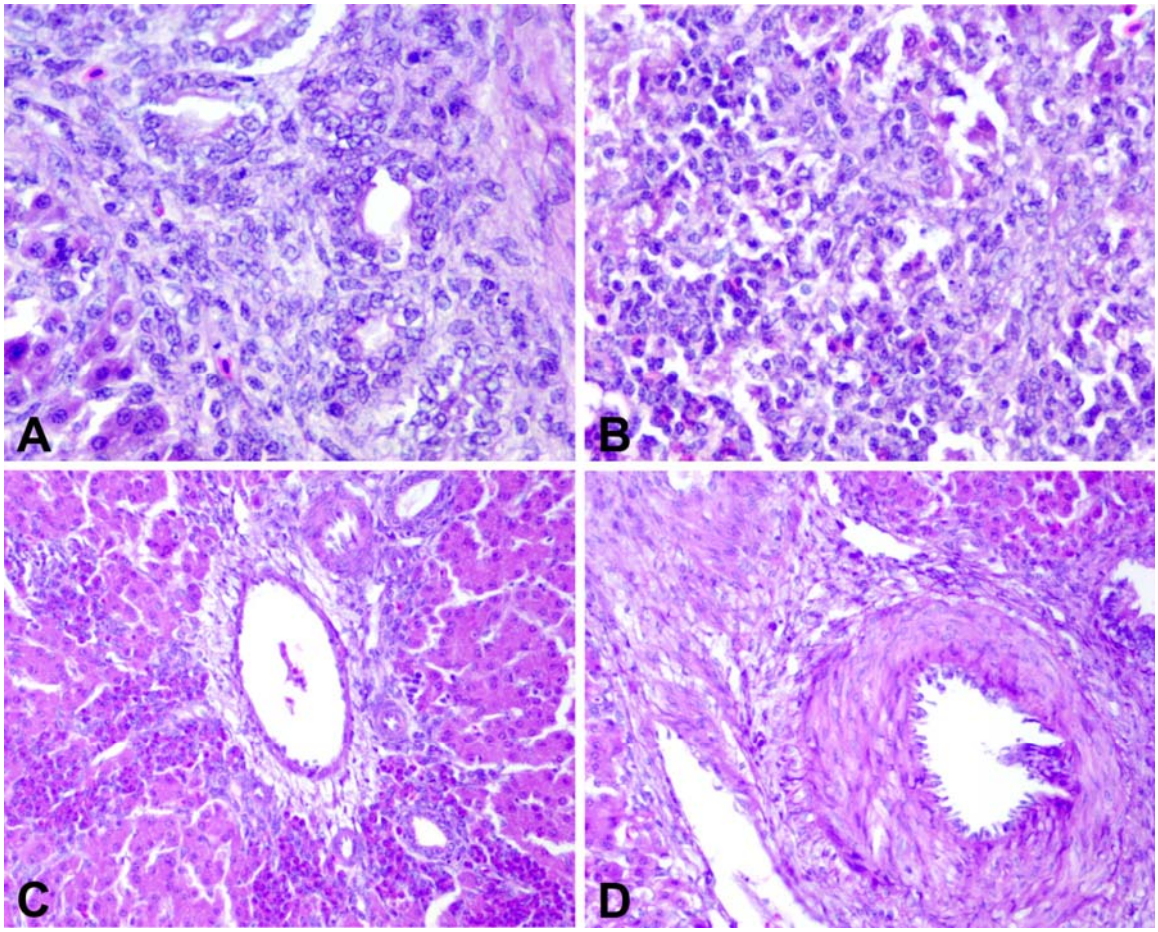

Fig. 1: Histopathological lesions in chicken liver (H&E Staining): A – strong proliferation of biliary ductules - type I (400x); B – oval cell hyperplasia – type III proliferation of biliary ductules (400x); C – Perivascular infiltration of myeloid cells (200x) ; D – swelling of arterial endothelial cells and fibromuscular dysplasia (200x).

DISCUSSION

Studies conducted upon the properties of propolis demonstrated that its addition into the diet protected hepatic tissue against adverse effects of various hepatotoxic factors, which leads to the formation of both regressive lesions (various types of degeneration and necrosis) and progressive lesions (cancers) (Banskota *et al*., 2000; Bazo *et al*., 2002; Aso *et al*., 2004; Bhadauria *et al*., 2008). This property of propolis has been attributed to its phenolic components (including flavonoids) and their anti-oxidizing effect, which ensured protection against lipid oxidation in cell membrane. Bhadauria and Nirala (2009) and Nirala and Bhadauria (2008) demonstrated in rats the protective effect of dietary an alcohol extract of propolis supplementation on regressive lesions in the liver and kidneys caused by the application of paracetamol. In the other experiment, Bhadauria *et al*. (2008) found a positive effect of propolis on liver damage caused by carbon tetrachloride. Results from recent studies, propolis demonstrated anticancer effects, not only against liver cancers, but also cancers of other organs including colorectal cancer and leukaemia (Bazo *et al*., 2002; Aso *et al*., 2004). The results obtained in the present research concerning the effects of propolis on liver morphology confirmed its beneficial influence, even when it is applied in small doses. The observed histopathological lesions were the least intensely expressed in broiler chickens of the experimental groups, and particularly in birds receiving propolis in the amount of 50 mg/kg of feed.

In the research performed, no significant changes were found in kidney morphology, or differences concerning this organ between birds of individual groups. Although this did not allow us to assess the effect of propolis on the morphology of the organ under discussion, the conclusion can be drawn that propolis in low doses certainly did not cause any pathological lesions in kidneys.

While assessing liver and kidney morphology, attention was also paid to the structure and the appearance of blood vessels. For the control groups, a microscopic analysis revealed irregularities in the morphological- pattern of artery walls, which were subject to clear induration, while the cytoplasm of myocytes showed increased acidophilia or it contained vacuoles. In chickens from both experimental groups (3 and 4), the degree of lesions in arteries was significantly lower than in birds from groups 1 and 2. Therefore, this fact indicated the beneficial effect of propolis supplementation on the morphological structures of blood vessels, which was also consistent with the reports of other researchers. Nadre *et al*. (2010) demonstrated an inhibitory effect of propolis on development of atherosclerosis in rabbits fed on the feed enriched with cholesterol. This effect was ascribed to the anti-oxidizing properties of propolis. This substance lowers the level of triacylglycerols, total cholesterol and LDL cholesterol in blood, increasing the level of HDL cholesterol. Thus, propolis prevented the occurrence of atherosclerotic lesions in arteries.

Conclusion: The results obtained in this study suggested that supplementation of propolis in the diet, even in low doses for the entire period of rearing broiler chickens, protected the liver of birds against pathological lesions, to which the birds were predisposed through an increasingly intensive method of poultry fattening. Propolis also demonstrated a protective effect towards the cardio-vascular system, inhibiting formation of pathological lesions in the blood vessel walls.

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