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THE ROLE OF DIETARY PROPOLIS ON ALBUMINS AND BILIRUBIN CONTENT IN CHICKENS

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ABSTRACT

The present study was designed to determinate the effect of propolis as a feed additive on the serum bilirubin and albumin content of female and male chickens. Broiler chickens hybrid Hubbard JV (n=500) were divided into five groups in each gender (control – C and four experimental groups E1 – E4). Experimental chickens received a propolis extract in feed mixture in various doses (E1 – 150 mg/kg; E2 – 450 mg/kg; E3 – 600 mg/kg; E4 – 800 mg/kg). The group that received feed without propolis addition served as the control. Contents of albumin and bilirubin were determined with spectrophotometer. Supplementation of the diet with propolis in the dose of 600 mg/kg significantly (P<0.05) increased albumin content in male chickens. Propolis addition to diets may be a source for antioxidant capacity in human and animals.

Keywords: Propolis, albumins, bilirubin, blood, chickens

INTRODUCTION

Reactive oxygen species (ROS) are important regulators for cellular and metabolic conditions. Molecular signalling is highly dependent on ROS. But depending on the amount of ROS production it might have toxic or protective effect (**Ullrich and Kissner, 2006**). Antioxidant molecules and agents are important players to influence the critical balance between production and elimination of reactive oxygen and nitrogen species (RONS) (**Elhaimeur *et al.*, 2002; Jansen and Daiber, 2012**). Bilirubin, a linear tetrapyrrole is the catabolic product of hemeproteins (**Nag *et al.*, 2009**), and it is consider as superior antioxidant (**Jansen and Daiber, 2012**). Free heme, which is toxic, is depredated via cleavage of its tetrapyrrollole ring by hemeoxygenase (**Maines, 2005**). Hemeoxygenase degrades heme to biliverdin, which is in then reduced by biliverdinreductase (BVR) to bilirubin. BVR mediates reduction of biliverdin to bilirubin being the much more potent antioxidant and subsequent oxidation of bilirubin by hydrogen peroxide back to biliverdin forming a catalytic antioxidant cycle that is driven by NADPH (Fig. 1), the reducing cofactor of BVR (**Sedlak and Snyder, 2004**). Bilirubin and biliverdin have reducing properties and are recognized as potent antioxidants (**Stocker *et al.*, 1987**). Especially lipophilic ROS such as lipid hydroperoxides and peroxyl radicals were described to feed this antioxidant cycle (**Sedlak and Snyder, 2004; Sedlak and Snyder, 2009**). Higher bilirubin concentrations are associated with a lower incidence of oxygen radical-mediated injury (**Hegyl*et al.*, 1994; Hammerman *et al.*, 1998**).

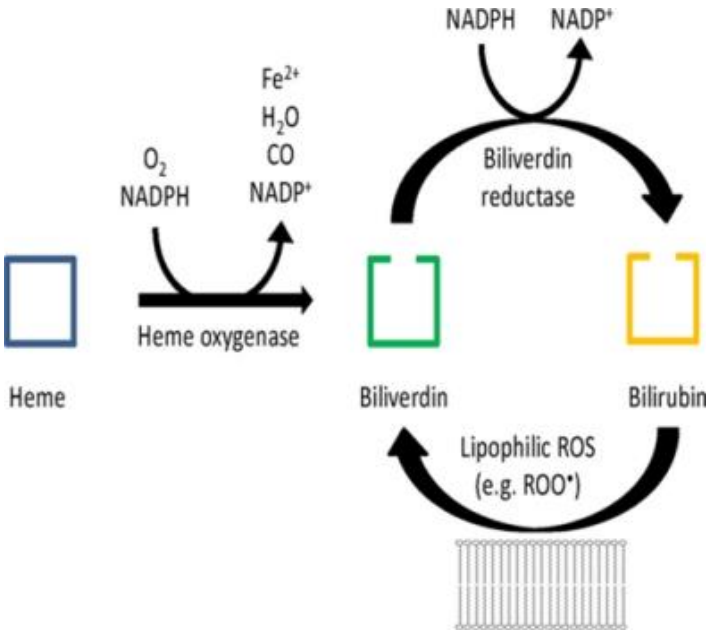

Figure 1 Scheme of the antioxidant redox cycle of bilirubin and other components (**Jansen *et al.*, 2012**, modified by **Sedlak and Snyder, 2004**).

It is not clear how bilirubin interacts with ROS to reduce its toxicity and on the other hand at elevated level increases ROS formation (**Nag *et al.*, 2009**). **Stojanov *et al.* (2013)** demonstrated that subjects with lower bilirubin concentration also have lower albumin concentration. Binding of bilirubin to albumin not only sequsters the molecule into a nontoxic form but also distributed the pigment throughout the entire circulation and extravascular space (**Brodersen, 1979; Stocker *et al.*, 1987**). Such albumin-bound bilirubin acts as an inhibitor of lipid peroxidation and can protect α-tocopherol from damage mediated by peroxyl radicals (**Neuzil and Stocker, 1993**).

Albumin, the most abundant protein in plasma, is a negative acute-phase protein and its concentration falls during the inflammatory process. In the inflammatory state, the activity of macrophages and other cells of immune system is enhanced, and macrophages show an increased free radical production which is implicated in disease development. Albumin may act as an indirect and sacrificial antioxidant and inhibits peroxidase free radical generation **(Era *et al.*, 1995).** Albumin has several other physiological functions. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key elements in the regulation of osmotic pressure and distribution of fluid between different compartments **(Roche *et al.*, 2008).** Many antioxidant activities of albumin result from its ligand-binding capacities **(Peters, 1996; Roche *et al.*, 2008)**.

Propolis is a resinous mixture collected from trees by the *Apis mellifera* bee. The principal components responsible for the biological activities of propolis samples are flavonoids, aromatic acids, diterpenic acids and phenolic compounds. Many studies confirmed beneficial effect of propolis on animal organism **(Aliciguzel *et al.*, 2003; Seven*et al.*, 2009; Kacaniova *et al.*, 2011; Petruska *et al.*, 2012)**.

The present study has investigated the dose and gender-dependent effect of propolis on serum bilirubin and albumin in broiler chickens.

MATERIAL AND METHODS

Animals and diets

The experiment was conducted on broiler chickens, hybrid Hubbard JV (n=500). Each group included 100 chickens (gender ratio 1:1). Chickens were randomly divided into five groups in each gender (control – C and four experimental groups E1 – E4) for female as well as for male group. Experimental chickens received a propolis extract in feed mixture in various doses (E1 – 150 mg/kg; E2 – 450 mg/kg; E3 – 600 mg/kg; E4 – 800 mg/kg). The group of chickens that received feed mixture without propolis inclusion served as the control. The propolis was obtained from pulverized propolis, mixed with 80% ethanol. The feeding period lasted 42 days. Chickens were fed *ad libitum* with complete feed mixture KKZ (Biofeeda.s., Kolarovo, Slovakia) as follows: KKZ HYD-01 (powdery form) from Day 1 till Day 21 and KKZ HYD-02 (granular form) from Day 22 till Day 42. Water was provided *ad libitum*. Ingredients and nutrient composition of diets are shown in Table 1.

Table 1 Diet composition of feed mixture KKZ HYD-01 and HYD-02.

Ingredient	KKZ HYD-01	KKZ HYD-02
Dry matter (g.kg^{-1})	917.3	913.3
Crude protein (g.kg^{-1})	211.3	199.7
Fat (g.kg^{-1})	25.5	23.0
Starch (g.kg^{-1})	413.0	434.8
Total sugar (g.kg^{-1})	49.5	31.7
ME (MJ)	11.689	11.555
Ca (g.kg^{-1})	12.121	8.207
P (g.kg^{-1})	7.833	6.834

Ca, calcium; P, phosphorus

Animals were kept in thermoneutral hall (from Day 1 33°C until 21°C at the end). In closed hall thermo aggregate was installed and experimental conditions with defined temperature and humidity were simulated by sensor. Simulated conditions were continually monitored using electronic recorder (Hivuss.r.o., Zilina, Slovak Republic). Animals were stabled in fattening hall with deep litter according to Welfare.

Chickens were healthy and their condition was judged as good at the commencement of the experiment. Conditions of animals care, manipulation and use corresponded with the instruction and were approved by local ethical commission.

Blood sampling and analyses

After 42 days of feeding chickens were slaughtered and blood samples (n=10 in each group, 5 males and 5 females) were obtained. The blood serum was separated from whole blood by centrifugation at 3000g for 30 min. The concentrations of serum bilirubin and albumins were analysed. The content of serum bilirubin and albumins were assayed by spectrophotometer Genesys 10 (using antioxidant RANDOX kits (Randox Labs., Crumlin, UK) according to the manufacturer's instructions.

Statistical analysis

SAS software and Sigma Plot 11.0 (Jandel, Corte Madera, USA) were used to conduct statistical analyses. One-way ANOVA was used to calculate basic statistic characteristics and to determine significant differences among the groups. Data presented are given as mean and standard deviation (SD). Differences were compared for statistical significance at the level $P<0.05$.

RESULTS AND DISCUSSION

Based on the concept that bilirubin and albumin molecules are good indicators of antioxidant status and can inform about the ability of organism to maintain antioxidant/oxidant balance, the present work investigated the changes in these parameters in chicken blood serum after propolis treatment. Many biological and antioxidant activities were reported for bee products (**Bankova *et al.*, 2009; Fatrcova-Sramkova *et al.*, 2008; Kacaniova *et al.*, 2011; Petruska *et al.*, 2012**). When bilirubin acts as an antioxidant, it is itself oxidized to biliverdin which is rapidly reduced by BVR to bilirubin (**Sedlak and Snyder, 2004; Sedlak and Snyder, 2009**).

The results of effect of propolis on bilirubin content in serum of broiler chickens are given in Figures 2-3. In males (Fig. 3), the values were more balanced in all doses in comparison with the female group (Fig. 2). In female the highest content of bilirubin was measured in E1 group and the lowest in the control. Statistical analyses showed no significant differences among the groups ($P>0.05$). In male the highest content of bilirubin was found in E4 group and similarly to the female group, the lowest content in the control, however without significant differences ($P>0.05$).

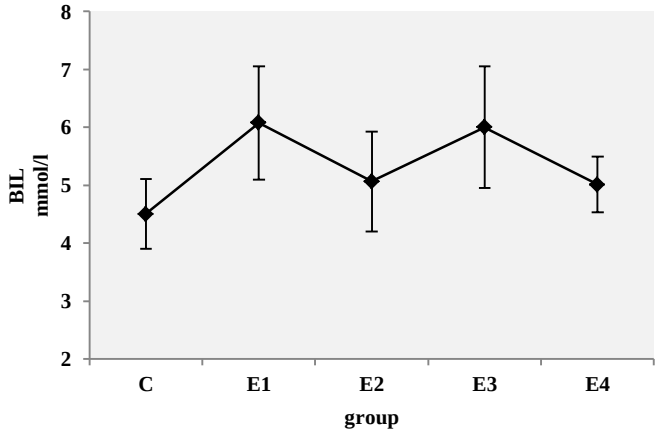

Figure 2 Effect of propolis on serum bilirubin content in female chickens
C – control group, E1 – E4 experimental groups with various doses of dietary propolis in feed mixture (E1 – 150 mg/kg; E2 – 450 mg/kg; E3 – 600 mg/kg; E4 – 800 mg/kg), BIL – bilirubin, differences were not significant ($P>0.05$), one-way ANOVA

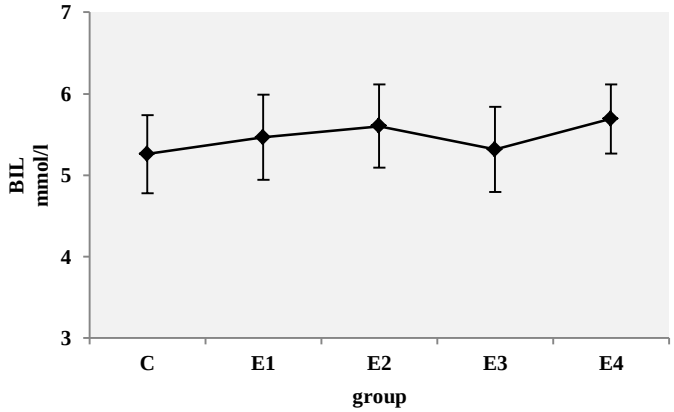

Figure 3 Effect of propolis on serum bilrubin content in male chickens
C – control group, E1 – E4 experimental groups with various doses of dietary propolis in feed mixture (E1 – 150 mg/kg; E2 – 450 mg/kg; E3 – 600 mg/kg; E4 – 800 mg/kg), BIL – bilirubin, differences were not significant ($P>0.05$), one-way ANOVA

Several lines of evidence suggest that high bilirubin serum concentrations are associated with increased total antioxidant capacity, and confer protection against oxidative stress-induced diseases **(Sedlak and Snyder 2009; Vitek, 2012).** In this paper the addition of propolis in various doses did not affect either the group of males or females in serum bilirubin content although the values in all experimental groups were slightly higher than those measured in the control. In our previous study addition of bee pollen had no effect on content of bilirubin in blood serum of rats **(Capcarova *et al.*, 2013).**

Changes in albumin content in chickens after propolis treatment are shown in Figures 4-5. Propolis had no effect on serum albumin in female chickens (Fig. 4) and differences among the groups remained insignificant (P>0.05). In males (Fig. 5) propolis addition caused increase of serum albumin in E1, E2 and E3 group, significantly (P<0.05) in E3 group when compared to the control. In E4 group the value decreased but it was still higher like the ones in the control group. Other results statistically did not differ (P>0.05) among the groups.

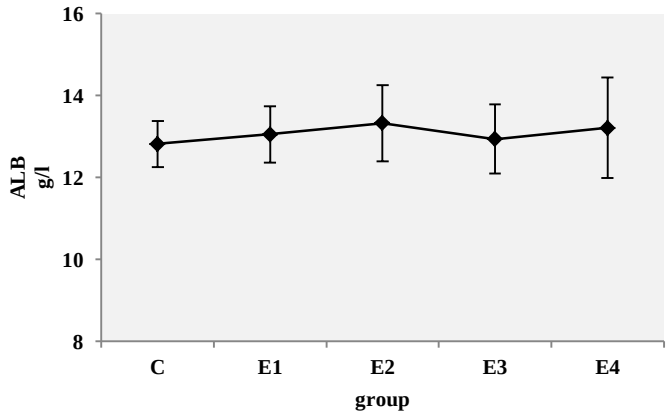

Figure 4 Effect of propolis on serum albumins content in female chickens
C – control group, E1 – E4 experimental groups with various doses of dietary propolis in feed mixture (E1 – 150 mg/kg; E2 – 450 mg/kg; E3 – 600 mg/kg; E4 – 800 mg/kg), ALB – albumins, differences were not significant (P>0.05), one-way ANOVA

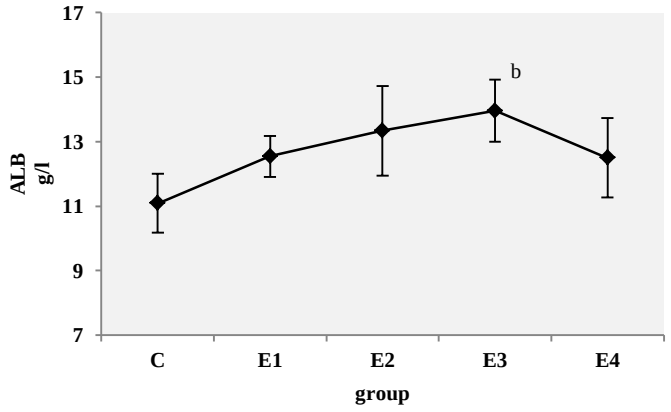

Figure 5 Effect of propolis on serum albumin content in male chickens
C – control group, E1 – E4 experimental groups with various doses of dietary propolis in feed mixture (E1 – 150 mg/kg; E2 – 450 mg/kg; E3 – 600 mg/kg; E4 – 800 mg/kg), ALB – albumin, a-b different letters mean significant differences at level P<0.05, one-way ANOVA

It was published that more than 70% of the free radical-trapping activity of serum was due to serum albumins (**Bourdon and Blache, 2001**). Copper and iron as transition metals are very potent to generate ROS after a reaction with oxygen. These ions can interact with hydrogen peroxide leading to the formation of the deleterious hydroxyl radical via Fenton reaction. Bound to proteins, copper and iron are generally less susceptible to participate in the Fenton reaction (**Roche *et al.*, 2008).**Our previous results revealed that bee pollen (0.5 g/kg of feed) significantly increased albumin content in rats (**Capcarova*et al.*, 2013).** Similar results in rabbits were published by **Attia *et al.* (2011)** with the dose of bee pollen 200 and 300 mg/kg body weight twice per week in the diet. In this study propolis had no effect on albumin content in female group but it increased the value of this parameter in male group of chickens in the dose of 600 mg/kg. It seems that males are more sensitive to propolis extract than females. The main flavonoid-like compound in propolis is caffeic acid phenethyl ester (CAPE) **(Lee *et al.*, 2007).** CAPE as active components of propolis exhibits antioxidant properties **(Koyu *et al.*, 2009).** The administration of propolis was concluded to exhibit antioxidant effect, and thereby to result in the alleviation of oxidative stress **(Kanbur *et al.*, 2009).**

CONCLUSION

In this study addition of propolis to the feed mixture for broiler chickens resulted in increase of serum albumin content in males when added in the dose of 600 mg/kg. Our results suggest a contribution of antioxidant properties of propolis. The beneficial effect of propolis seems to be dose and gender dependent.

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REFERENCES

ALICIGUZEL, Y., OZEN, I., ASLAN, M., KARAYALCIN, U. 2003. Activities of xanthine oxidoreductase and antioxidant enzymes in different tissues of diabetic rats. *Journal of Laboratory and Clinical Medicine***,** 142, 172-177.

ATTIA, Y.A., AL-HANOUN, A., BOVERA, F. 2011. Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months. *Journal of Animal Physiology and Animal Nutrition* (Berl.), 95, 17-26.

BANKOVA, V. 2009. Chemical diversity of propolis makes it a valuable source of new biologically active compounds. *Journal of Api Product and Api Medical Sciences*, 1(2), 23-28.

BOURDON, E., BLACHE, D. 2001. The importance of proteins in defence against oxidation. *Antioxidant and Redox Signaling*, 3, 293-311.

BRODERSEN, R. 1979. Binding of bilirubin to albumin: Implications for prevention of bilirubin encephalopathy in the newborn. *Critical Reviews in Clinical Laboratory Sciences*,11, 305-399.

CAPCAROVA, M., KOLESAROVA, A., KALAFOVA, A., GALIK, B., SIMKO, M., JURACEK, M., TOMAN, R. 2013. The role of dietary bee pollen in antioxidant potential in rats. *Eurasian Journal of Veterinary Sciences*, 29, 133-137.

ELHAIMEUR, F., COURDEROT-MASUYER, C., NICOL, L., GUYON, C., RICHERT, L., BERTHELOT, A., 2002. Dietary vitamin C supplementation decreases blood pressure in DOCA-salt hypertensive male Sprague-Dawley rats and this is associated with increased liver oxidative stress. *Molecular and Cellular Biochemistry*, 237, 77-83.

ERA, S., KUWATA, K., IMAI, H., NAKAMURA, K., HAYASHI, T., SOGAMI, M. 1995. Age related change in redox state of human serum albumin. *BiochimicaetBiophysicaActa*, 1247, 12-16.

FATRCOVA-SRAMKOVA, K., NOZKOVA, J., MARIASSYOVA, M., KACANIOVA, M., DUDRIKOVA, E., 2008. Content of polyphenols and antiradical activity of bee pollen. *Chemicke Listy*, 102, 265-267.

HAMMERMAN, C., GOLDSTEIN, R., KAPLAN, M., ERAN, M., GOLDSMIDT, D., ELDERMAN, A.I., GARTNER, L.M. 1998. Bilirubin in the premature: toxic waste or natural defence? *Clinical Chemistry*, 44, 2551-2553.

HEGYL, T., GOLDIE, E., HIATT, M. 1994. The protective role of bilirubinin oxygen-radical disease of the preterm infant. *Journal of Periantology*, 14, 296-300.

JANSEN, T., DAIBER, A. 2012. Direct antioxidant properties of bilirubin and biliverdin. Is there a role for biliverdinreductase? *Frontiers in Farmacology*, 3, Article 30, 10 pages.

KACANIOVA, M., FATRCOVA-SRAMKOVA, K., NOZKOVA, J., MELICH, M., KADASI-HORAKOVA, M., KNAZOVICKA, V., FELSÖCIOVA, S., KUNOVA, S., MARIASSYOVA, M. 2011. Antiradical activity of natural honeys and antifungal effect against *Penicillium* genera. *Journal of Environmental Science and Health, Part B*, 46, 92-96.

KANBUR, M., ERASLAN, G., SILICI, S. 2009. Antioxidant effect of propolis against exposure to propetamphos in rats. *Ecotoxicology and Environmental Safety*, 72, 909-915.

KOYU, A.,OZGUNER, F.,YILMAZ, H.,UZ, E.,CESUR, G.,OZCELIK, N. 2009. The protective effect of caffeic acid phenethyl ester (CAPE) on oxidative stress in rat liver exposed to the 900 MHz electromagnetic field. *Toxicology and Industrial Health*, 25, 429-434.