

## EFFECTS OF BORNEOL ON BLOOD CHEMISTRY CHANGES IN CHICKENS

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*It is well established that borneol may be alter bone metabolism. The aim of the study was to investigate the effects of feeding diets supplemented with borneol on blood biochemistry of chickens. Forty chicks of a commercial strain ISA Brown were divided into five groups with 8 birds in each and fed diets supplemented with 0%, 0.1%, 0.05% or 0.025% borneol dissolved in sunflower oil from hatching up to week 11. The concentrations of potassium and magnesium were found to be significantly higher in chickens fed the diet supplemented with 0.1% and 0.025% borneol, respectively. The blood plasma levels of total protein and glucose in chickens treated with 0.05% borneol were significantly lowered. Feeding of diets supplemented with borneol significantly increased levels of bilirubin, cholesterol and total lipids in blood plasma compared to the control group supplemented with sunflower oil only. The present study shows that addition of borneol to the diets can influence some parameters of mineral, lipid and protein metabolism in chicken blood plasma.*

*Key words: borneol, essential oil, metabolism, poultry*

### INTRODUCTION

Essential oils are volatile natural complex compounds formed by aromatic plants as secondary metabolites. They are usually obtained by steam or hydro-distillation and the extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant tissue, age and vegetative cycle (Angioni *et al.*, 2006). Essential oils contain about 20 – 60 components at different concentrations. They are characterized by two or three major components (mostly monoterpenes) at fairly high concentrations (20 to 70%) compared to other components present in trace amounts. For example, carvacrol (30%) and thymol (27%) are major components of the *Origanum compactum* essential oil and 1.8 cineole (50% and 36.1%) of the *Cinnamomum camphora* essential oil and *Rosmarinus officinalis* essential oil, respectively (Debersac *et al.*, 2001; Bakkali *et al.*, 2008).

Generally, these major components determine the biological properties of the essential oil which can result from complex functions between its constituents and produce both synergetic and antagonistic responses between the components. Understanding such interactions is important in comparing species on the basis of chemical composition.

The results of numerous studies showed that biologically active components in essential oils exhibit cytotoxic (Bozin *et al.*, 2007), antioxidant (Faix *et al.*, 2007, Faixová and Faix, 2008a), anticarcinogenic (Alexandrov *et al.*, 2006) and cognition-enhancing properties (Pilija *et al.*, 2005). Some of them also lower elevated blood sugar levels (Ziegenfuss *et al.*, 2006, Faixová and Faix, 2008b) and favorably alter systolic blood pressure (Preuss *et al.*, 2006).

Borneol is a monoterpene synthesized by plant organs of herbs including leaves of rosemary *Rosmarinus officinalis* L. (5.4%) (Debersac *et al.*, 2001), wild-growing sage *Salvia officinalis* L. from Monte Negro (4.4%) (Damjanoviae – Vratnica *et al.*, 2008) or *Artemisia feddei* (5.08%) (Cha *et al.*, 2007). Many reports showed that borneol possesses anti-inflammatory (Tung *et al.*, 2008), antioxidant and antimicrobial properties (Kelen and Tepe, 2008). The results of *in vitro* studies suggested that borneol could inhibit bone resorption (Dolder *et al.*, 2006) and cholinesterase enzyme activity (Savelev *et al.*, 2003).

The objectives of the current study were to determine effects of borneol on plasma biochemical parameters of mineral, lipid and protein metabolism in chickens.

## MATERIAL AND METHODS

### *Animal, diets and treatment*

Forty chickens of a commercial strain ISA Brown were randomly divided at the day of hatching into five groups (n=8 in each). All birds received the basal diet HYD-04/a for 7 weeks and then they were fed a basal diet HYD-04/b for the 4 following weeks (BIOFER, Vel'ký Šariš, Slovakia). The composition of the diets is given in Table 1. The five types of diet included control I (basal diets); control II (basal diet + sunflower oil); and experimental (basal diets + sunflower oil supplemented with 0.1%, 0.05% and 0.025% borneol) (Aldrich). Borneol was dissolved in sunflower oil; sunflower oil content in the diets was 1%. All birds had free access to water and feed.

The chickens were placed in large pens with wood shavings. Rearing of chickens started with a light regimen of 23 h light to 1 h dark and lasted for 4 weeks. The initial room temperature 32–33 °C was reduced weekly by 1 °C to a final temperature of 28 °C. The experiment was carried out in accordance with the established standards for experimental animals and birds. The protocol was approved by the local ethical and scientific authorities.

Table 1. Composition of diets fed to chickens during the entire experiment. HYD-04/a (7 weeks), HYD-04/b (4 weeks)

Component	HYD-04/a	HYD-04/b
Metabolizable energy (MJ.kg <sup>-1</sup> )	11.9	11.5
Total nitrogen (g.kg <sup>-1</sup> )	195.0	175.0
Lysine (g)	10.5	8.0
Methionine and Cystine (g.kg <sup>-1</sup> )	7.5	7.0
Methionine (g.kg <sup>-1</sup> )	4.0	3.5
Calcium (g.kg <sup>-1</sup> )	8.0	8.0
Phosphorus (g.kg <sup>-1</sup> )	5.0	5.0
Natrium (g.kg <sup>-1</sup> )	1.2 – 2.5	1.2 – 2.5
Linoleic acid (g.kg <sup>-1</sup> )	8.0	8.0

Vitamin-mineral premix provided *per* kg complete diets HYD-04/a and HYD-04/b: vitamin A, 10 000 and 8 000 UI, respectively; cholecalciferol, 2 000 and 1 500 UI, respectively; vitamin E ( $\alpha$ -tocopherol), 15 and 12 mg, respectively; vitamin K, 2 mg; tiamin, 4 mg; riboflavin, 4 mg; pyridoxine, 4 mg; cobalamine, 10 mg; biotin, 0.2 mg; folic acid, 1 mg; niacin 40 mg; betain, 200 mg; iodine, 0.89 mg; pathotenic acid, 15 mg; selenium, 0.1 mg; cobalt, 0.2 mg; manganese, 70 and 50 mg, respectively; iron, 60 mg; copper, 6 mg; zinc, 50 mg.

#### Sample collection

At 11 weeks of age, chickens were anaesthetized by intraperitoneal injection of xylazine (Rometar 2%, SPOFA, Czech Republic) and ketamine (Narkamon 5%, Czech Republic) at doses of 0.6 and 0.7 mL/kg of body weight, respectively. After laparotomy, blood was collected into heparinized tubes by intracardial puncture and centrifuged for plasma specimens at 1 180 g for 15 min. Blood samples were frozen and stored at -65 °C.

#### Sample analysis

Biochemical parameters were determined by the colorimetric method using spectrophotometric kits (Randox lab, Ardmore, UK). Plasma calcium concentration was determined by the method of Ray Sarkar and Chauchan (1967); magnesium was measured by the method of Mann and Yoe (1957); potassium was measured by the method of Hilmann and Beyer (1967); total protein concentration was determined by the method of Doumas *et al.* (1981); triglycerides concentration was measured by the method of Koditschek and Umbreit (1969).

Blood plasma concentrations of phosphorus, uric acid, bilirubin, glucose, cholesterol, total lipids and alkaline phosphatase (E.C 3.1.3.1) (ALP) were determined using REFLOTRON spectrophotometer autoanalyzer (Boehringer Mannheim, Germany).

*Statistical analysis*

Statistical analysis was done by one-way analysis of variance (ANOVA) with the *post hoc* Tukey multiple comparison test. The results are given as means  $\pm$  S.E.M.

## RESULTS

Chickens did not show any visible clinical signs of borneol toxicosis during the experiment. Eleven-week-intake of diets supplemented with borneol resulted in increased concentrations of potassium and magnesium at 0.1 and 0.025% supplementation compared to the control group with sunflower oil, respectively. Plasma concentration of calcium was lower at 0.1% borneol concentration. The concentration of phosphorus was not changed by the diets. Diets with different supplementation of borneol had different effects on the biochemical parameters in blood plasma. Addition of borneol to the diet led to a significantly decreased level of uric acid at 0.1% concentration of borneol while 0.05% borneol significantly increased the level of uric acid in blood plasma.

The blood plasma level of bilirubin, cholesterol, activity of alkaline phosphatase and total lipids were significantly higher than in the control group. Plasma triglycerides concentrations were not affected by the diets (Table 2).

Table 2. Parameters of mineral, lipid and protein metabolism in blood plasma of chickens. Control I – basal diet; Control II – basal diet + sunflower oil; mean  $\pm$  S.E.M., n = 8

	Control I	Control II	Borneol 0.025 %	Borneol 0.05 %	Borneol 0.1 %
Potassium (mmol/L)	7.1 $\pm$ 0.13	6.9 $\pm$ 0.05 <sup>a</sup>	7.2 $\pm$ 0.1	7.4 $\pm$ 0.1	7.4 $\pm$ 0.1 <sup>b</sup>
Calcium (mmol/L)	2.2 $\pm$ 0.1	2.4 $\pm$ 0.1 <sup>a</sup>	2.1 $\pm$ 0.1	2.2 $\pm$ 0.03	2.1 $\pm$ 0.1 <sup>b</sup>
Magnesium (mmol/L)	0.96 $\pm$ 0.02 <sup>a</sup>	0.89 $\pm$ 0.04 <sup>a</sup>	1.4 $\pm$ 0.1 <sup>b</sup>	1.2 $\pm$ 0.1	1.0 $\pm$ 0.05
Phosphorus (mmol/L)	1.0 $\pm$ 0.1	1.3 $\pm$ 0.05	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1
Alkaline phosphatase ( $\mu$ kat/L)	14.2 $\pm$ 0.5 <sup>a</sup>	22.3 $\pm$ 1.2 <sup>bde</sup>	18.6 $\pm$ 0.9 <sup>bce</sup>	21.6 $\pm$ 1.2 <sup>e</sup>	25.4 $\pm$ 0.9 <sup>de</sup>
Total protein (g/L)	70.1 $\pm$ 0.9 <sup>a</sup>	72.3 $\pm$ 0.7 <sup>a</sup>	70.5 $\pm$ 0.4 <sup>a</sup>	62.3 $\pm$ 1.2 <sup>b</sup>	72.5 $\pm$ 1.5 <sup>a</sup>
Uric acid ( $\mu$ mol/L)	206 $\pm$ 8 <sup>a</sup>	222 $\pm$ 9 <sup>a</sup>	221 $\pm$ 6 <sup>a</sup>	352 $\pm$ 10 <sup>b</sup>	154 $\pm$ 10 <sup>c</sup>
Bilirubin ( $\mu$ mol/L)	4.4 $\pm$ 0.4 <sup>a</sup>	3.8 $\pm$ 0.2 <sup>a</sup>	4.5 $\pm$ 0.3 <sup>ab</sup>	5.9 $\pm$ 0.3 <sup>b</sup>	5.6 $\pm$ 0.1 <sup>b</sup>
Cholesterol (mmol/L)	3.0 $\pm$ 0.1 <sup>a</sup>	3.2 $\pm$ 0.1 <sup>a</sup>	5.3 $\pm$ 0.4 <sup>b</sup>	4.6 $\pm$ 0.1 <sup>bc</sup>	4.3 $\pm$ 0.3 <sup>c</sup>
Triglycerides (mmol/L)	2.6 $\pm$ 0.1	2.7 $\pm$ 0.1	2.5 $\pm$ 0.1	2.9 $\pm$ 0.1	2.5 $\pm$ 0.04
Total lipids (mmol/L)	3.7 $\pm$ 0.1 <sup>ad</sup>	4.3 $\pm$ 0.1 <sup>bde</sup>	4.8 $\pm$ 0.1 <sup>ce</sup>	4.1 $\pm$ 0.1 <sup>de</sup>	4.3 $\pm$ 0.1 <sup>e</sup>
Glucose (mmol/L)	17.8 $\pm$ 0.4 <sup>a</sup>	18 $\pm$ 0.2 <sup>a</sup>	12.4 $\pm$ 0.5 <sup>b</sup>	11.3 $\pm$ 0.4 <sup>b</sup>	15.4 $\pm$ 0.4 <sup>c</sup>

Different superscripts indicate significant differences within a row (P<0.05).

## DISCUSSION

Our results give evidence on the effects of borneol concentrations tested on biochemical parameters in chickens.

Feeding diets supplemented with 0.1 % of borneol had reducing effect on plasma concentration of calcium. Our results correspond to the study of Mühlbauer *et al.* (2003). They reported that dried leaves of sage strongly inhibit bone resorption. Therefore, they tested several common herbs rich in essential oils, as well as their monoterpene components. They investigated that the monoterpene borneol can intensively and reversibly inhibit bone resorption in rats both *in vivo* and *in vitro*. Similarly, Dolder *et al.* (2006) reported in their study that monoterpenes inhibit bone resorption *in vivo* through a direct effect on the formation of osteoclasts acting mainly on hemopoietic cells. Recently, Jeong *et al.* (2008) evaluated the anabolic activities of 89 natural compounds by measuring the amount of newly synthesized calcium in the differentiation of mouse osteoblastic MC3T3-E1 subclone 4 cells. They found that a low concentration of 3-carene (a bicyclic monoterpene in essential oils) extracted from pine trees, significantly stimulated the activity and expression of alkaline phosphatase, an early phase marker of osteoblastic differentiation. Later it dramatically promoted the induction of calcium in a dose-dependent manner. The authors suggested that the use of natural additives to the diet including essential oils could have a beneficial effect on bone health. Although these studies report contradictory data, they should not be considered to be reciprocally exclusive, as bone metabolism is a complex multifactorial process and it is known that stimuli can give rise to anabolic or catabolic effects depending on dosage, timing and site of application.

Dietary supplementation of borneol to chickens for 11 weeks caused a significantly lower blood plasma glucose concentration. A number of spices and herbs have a long history of traditional use in treating elevated blood sugar levels. For example Jarvill-Taylor *et al.* (2001) reported that cinnamon stimulated glucose uptake, glycogen synthesis, and activated glycogen synthase in 3T3-L1 adipocytes. Later studies of Bakirel *et al.* (2008) showed that ethanolic extracts of rosemary leaves (*Rosmarinus officinalis* L.) lowered blood glucose in normoglycemic and glucose-hyperglycemic rabbits.

In the present experiment, there was a significant increase in plasma total bilirubin in birds fed diets supplemented with 0.1% and 0.05% borneol. An increase in total bilirubin level in the plasma in the current study might be explained by an abnormality of uridine diphosphate glucuronyltransferase enzyme (UDP-glucuronyltransferase). There are several reports concerning an inhibition of hepatic UDP glucuronyltransferase by borneol. Siraki *et al.* (2005) found out that borneol inhibited glucuronidation of non steroidal anti-inflammatory drugs in isolated rat hepatocytes. Further evidence supporting these is given by Gelal *et al.* (1999) and Li *et al.* (2003).

Increased concentration of total lipids could point out cell damage. Lima *et al.* (2004) observed toxic effects of essential oils on isolated rat hepatocytes. They confirmed increased lipid peroxidation and cell damage at high doses. In contrast to results by Lima *et al.* (2004), plasma lipid concentrations of female broilers fed

thymol, cinnamaldehyde and commercial preparation of essential oil components (CRINA Poultry) were not affected in the study of Lee *et al.* (2003).

In conclusion, our results indicate that borneol has a significant effect on liver and bone metabolism of chickens.

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**UTICAJ BORNEOLA NA PROMENE VREDNOSTI BIOHEMIJSKIH PARAMETARA U  
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## SADRŽAJ

Cilj ovih ispitivanja je bio da se utvrdi uticaj smeša za ishranu pilića u koje je dodavan borneol na vrednosti biohemijskih parametara krvi. U ogled je bilo uključeno 40 pilića soja ISA Brown podeljenih u pet grupa po osam jedinki. Jedinke prve kontrolne grupe su hranjene standardnom krmnom smešom a druge smešom sa dodatkom suncokretovog ulja. Jedinke prve ogledne grupe dobijale su borneol u koncentraciji od 0,1%, druge 0,05% i treće 0,025% umešan u suncokretovo ulje od izleganja do 11-te nedelje.

Kod jedinki prve i treće ogledne grupe dokazane su veće koncentracije kalijuma i magnezijuma u krvi. Koncentracije ukupnih proteina i glukoze bile su značajno niže u drugoj oglednoj grupi. Sve grupe hranjene smešama sa dodatkom borneola, imale su značajno povećane koncentracije bilirubina, holesterola i ukupnih lipida u poređenju sa kontrolama.

Naši rezultati ukazuju da dodatak borneola krmnim smešama za ishranu pilića može uticati na vrednosti nekih parametara metabolizma proteina, masti i minerala.