

Effect of *Cinnamomum zeylanicum* Essential Oil on Antioxidative Status in Broiler Chickens

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Abstract

The experiment was conducted to investigate the effects of *Cinnamomum zeylanicum* essential oil on antioxidant status of chickens. Thirty-two female Ross 308 hybrid broilers were fed one of four diets supplemented with 0%, 0.1%, 0.05% and 0.025% of essential oil for 38 days. Blood, liver, kidney and duodenal epithelium were collected for the subsequent evaluation of antioxidant status. Feeding of adiet supplemented with 0.1% of essential oil significantly decreased the concentration of malondialdehyde (MDA) in plasma and duodenal mucosa in comparison with the control group (0%). The activities of glutathione peroxidase (GPx) were significantly higher in blood of chicks fed the diet containing 0.1% of essential oil. Diets containing 0.05% and 0.025% of essential oil reduced alanine amino transferase (ALT) activity in plasma in comparison with the control group. Blood phagocytic activity significantly increased in chickens fed the diet supplemented with 0.1% and the index of phygocytic activity was affected by the diet containing 0.025% of essential oil in comparison with the control group. The present investigation shows that *Cinnamomum zeylanicum* essential oil exhibits a significant antioxidant activity in fattening chickens and can be used as a source of antioxidant in dietary supplement.

Essential oil, lipid peroxidation, antioxidant enzymes, phagocytosis

Cinnamomum zeylanicum is one of the oldest herbal medicines known, having been mentioned in Chinese texts as long as 4,000 years ago. It is often used for medicinal purposes due to its unique properties. The essential oil from *Cinnamomum zeylanicum* bark is rich in trans-cinnamaldehyde with antimicrobial effects against animal and plant pathogens, food poisoning and spoilage bacteria and fungi (Mastura et al. 1999). Until now, more than 300 volatiles were found as constituents of essential oils of cinnamon. The essential oil derived from cinnamon leaves is rich in eugenol, that from the roots in camphor and that from the buds shows a high amount of sesquiterpenes (α -bergamotene and α -copaene) (Jayaprakasha et al. 2002). It has been established that the oils and extracts from cinnamon possess a distinct antioxidant activity, which is especially attributed to the presence of phenolic and polyphenolic substances (Jayaprakasha et al. 2006; Tomaino et al. 2005).

These main properties of cinnamon are astringent, warming, stimulating, carminative, antiseptic, antifungal, antiviral, blood purifying, and aiding digestion. All of these properties of cinnamon make it a good medicinal plant. Cinnamon is more often used as a non-essential addition to other remedies than as a remedy by itself. The medicinal effects of cinnamon oil are very powerful, and there are many uses for it. However, principally it is used as an aromatic to cover the unpleasant taste of other drugs. The various terpenoids found in the spice essential oil are thought to be the reason for cinnamon's medicinal properties. Eugenol and cinnamaldehyde are two very important terpenoids found in cinnamon. Cinnamaldehyde and cinnamon oil vapors act as potent antifungal agents. The diterpenes found in the cinnamon oil have shown antiallergenic activity. The numerous uses of cinnamon as a medicinal herb imply the widespread appreciation of its healing

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effects by herbalists worldwide. Unfortunately, often there is no scientific research to backup the claims that cinnamon does in fact have healing powers. Along with the medicinal effects come the side effects and interactions that medicinal cinnamon causes. Recent trends and developments in the area of animal nutrition have been characterized by an increasing interest in the potential impact of plants, herbs and spices on the immune function and antioxidant status of humans and animals. Research activities have been intensified; however, understanding of the specific mode of action and the functional aspects of phytogetic additives still needs many more in depth studies. *Cinnamomum zeylanicum* essential oil was tested for an effect on intestinal cell viability (Fabian et al. 2006). Antioxidant activities of volatile extracts isolated from cinnamon were evaluated by various *in vitro* assays (Lee and Shibamoto 2002). The contents of glutathione (GSH) and lipid-conjugated dienes were studied in rats fed a high-fat diet along with cinnamon and it was reported that cinnamon stimulates the activity of antioxidant enzymes (Dhuley 1999). In addition, the effect of cinnamate, a phenolic compound found in cinnamon bark and other plant materials, on lipid metabolism and antioxidant enzyme activities in rats fed a high cholesterol diet has been studied and indicated that cinnamon suppresses lipid peroxidation via the enhancement of hepatic antioxidant enzyme activities (Lee et al. 2003). In a study of the wound healing action of an ethanol extract of cinnamon, the significant increase in wound healing was attributed to the antioxidant activity (Kamath et al. 2003). Cinnamon constituents possess antioxidant action and may prove beneficial against free radical damage to cell membranes (Dragland et al. 2003). Cinnamon has a marked antioxidant potential and may be beneficial in alleviating the complications of many illnesses related to oxidative stress in humans (Ranjbar et al. 2006). Cinnamon extracts are used regularly as food antioxidants, also improving food palatability (Mancini-Filho et al. 1998).

The aim of this experiment was to investigate the effects of diets supplemented with *Cinnamomum zeylanicum* essential oil on some indicators of blood and tissue antioxidant status and blood phagocytic activity in fattening chickens.

Materials and Methods

Animals, diets and treatments

The experimental protocol was approved by the local Ethics Committee; the principles of animal protection were strictly followed. Thirty-two 1-day-old female Ross 308 hybrid broilers were obtained from the breeding company LP (Parovske Haje a.s., Slovakia) and divided into four groups. Each group contained 8 birds that were placed in pens (2.5 m × 1.5 m) with wood shavings. Temperature and lighting regimens were in accordance with the recommendation of the breeder. Rearing of the chickens started with a lighting 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. Birds were fed a basal diet obtained from Agrokonzult s.r.o. (Nove Zamky, Slovakia). The composition of this diet is presented in Table 1. All birds had free access to water and feed. The control group was fed a basal diet; the second, third and fourth groups were fed the same basal diets supplemented with 0.1%, 0.05% and 0.025% essential oil, respectively. *Cinnamomum zeylanicum* essential oil was purchased from Calendula (Calendula a.s., Nova Lubovna, the Slovak Republic), certificate of quality No. 610. Essential oil was diluted with sunflower oil and the final concentration of sunflower oil was 1% of diet.

Sample collections

At 38 days of age, 8 randomly chosen chickens from each group were anaesthetised by intraperitoneal injection of xylazine (Rometa 2%, SPOFA, Czech Republic) and ketamine (Narkamon 5%, SPOFA, Czech Republic) at 0.6 and 0.7 ml·kg⁻¹ body weight, respectively. After laparotomy, blood was collected into heparinised tubes by intracardial puncture and centrifuged for plasma specimens at 1 180 g for 15 min. Samples of blood and plasma for analysis were frozen and stored at -65 °C. Following euthanasia, samples of liver, kidney and duodenal mucosa tissues were collected and stored also at -65 °C until analysis.

Sample analysis

The activity of blood glutathione peroxidase (GPx, EC 1.11.1.9) was determined using the method of Paglia and Valentine (1967) with a Ransel kit (Randox, UK). To analyse the activities of GPx in liver, kidney and duodenal mucosa, pieces of tissue were homogenised in phosphate buffer saline with pH 7.4 and

Table 1. Composition of basal diets fed to broilers during the entire experiment

Component	Growing period		
	0-18 days HYD-01 (%)	19-31 days HYD-02 (%)	32-38 days HYD-03 (%)
Wheat (ground)	34.84	30.00	30.00
Maize (ground)	35.00	41.40	43.00
Soybean extracted ground meal	21.00	22.00	21.60
Fish meal	5.00	2.00	-
Calcium carbonate	1.00	1.00	1.10
MCP	0.80	0.70	0.90
Sodium chloride	0.32	0.35	0.35
Lysine	0.18	0.22	0.25
Methionine	0.25	0.25	0.24
Threonine	0.05	0.05	0.05
Cycostat 66G	0.05	-	-
Bergafat 306	1.00	1.47	2.00
KWD	0.01	0.01	0.01
Sacox 12%	-	0.05	-
Euromix Hyd 01, 02, 03	0.50	0.50	0.50
Calculated analysis			
Metabolic energy (MJ)	12.09	12.24	12.34
Total nitrogen (g)	208.90	191.67	176.86
Lysine (g)	11.94	10.93	10.02
Methionine (g)	6.05	5.55	5.06
Linoleic acid (g)	12.75	13.69	13.99
Phosphorus (g)	5.41	4.22	3.75
Calcium (g)	8.24	7.20	7.30

MCP-monocalcium phosphate (227g P/kg MCP, 160g Ca/kg MCP), Cycostat 66G -coccidiostaticum, Bergafat 306-energy substances on fat base, KWD-multienzymatic preparation, Saxox 12%-ionophor (Natrium salinomycinat), Euromix Hyd 01, 02, 03-mixture of vitamins and minerals.

containing 1 mM Na₂ EDTA.2H₂O. Homogenates were centrifuged at 13 680 g at 4 °C for 20 min. Enzyme activity in the supernatant was measured by monitoring oxidation of NADPH + H⁺ at 340 nm. Tissue samples of liver, kidney and duodenal mucosa for malondialdehyde (MDA) determination were homogenised with deionised distilled water and 50 ml of butylated hydroxytoluene. The MDA concentrations in homogenates were measured by the modified fluorimetric method in accordance with Jo and Ahn (1998). Haemoglobin (Hb) content of blood and superoxide dismutase (SOD, EC 1.15.1.1) activities (Arthur and Boyne 1985) in the erythrocytes were analysed using kits from Randox, UK. The protein concentrations in the tissues examined were measured by the spectrophotometric method of Bradford (1976). The γ -glutamyl transferase (GGT, EC 2.6.1.2) activity was measured using the kit from Randox, UK, by the method of Szasz (1969). Catalase activity was assayed by the method of Pippenger et al. (1998). Ellman's method (1958) was used to determine -SH groups. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with the use of the method by Reitman and Frankel (1957). For spectrophotofluorimetric measurement was used spectrophotofluorimeter (Shimadzu, RF-540) and for spectrophotometric measurements UV VIS spectrophotometer (Specord UV VIS, Carl Zeiss Jena).

The phagocytic activity was measured by direct counting procedure using microspheric hydrophilic particles (MSHP). Ingestion of MSHP particles by polymorphonuclear cells (PMNs) was determined by a modified test described by Vetvicka et al. (1982). One-hundred cells were examined to determine the relative number of white cells containing at least three engulfed particles (phagocytic activity) and the index of phagocytic activity (number of engulfed particles/number of neutrophils and monocytes). The percentage of phagocytic cells was evaluated using an optical microscope, by counting PMN up to 100.

Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) with the *post hoc* Tukey multiple comparison test using GraphPad Software (USA). The results are given as means \pm SEM.

Results

Chickens for fattening did not show any visible clinical changes during the whole experiment. As shown in Table 2, the concentration of MDA was significantly lower in duodenal mucosa in the group supplemented with 0.1% of essential oil only. MDA concentration in liver and kidney tissues were not significantly affected by diets. The GPx activity in the liver was significantly higher only in the group of birds fed the diet supplemented with 0.05% of essential oil compared to the control and both experimental groups. The GPx activity in the kidneys was significantly higher only in the group supplemented 0.05% of essential oil compared to the control group only.

Table 2. Concentration of MDA and activity of GPx in tissues of broilers fed diets supplemented with different concentrations of *Cinnamomum zeylanicum* essential oil (means \pm SEM; n = 8 in each group)

Indices	Control	0.10%	0.05%	0.025%
Duodenal mucosa				
MDA ($\mu\text{g}\cdot\text{mg}^{-1}$ protein)	0.54 \pm 0.03 ^a	0.37 \pm 0.03 ^a	0.51 \pm 0.04	0.43 \pm 0.05
GPx (nkat $\cdot\text{mg}^{-1}$ protein)	1.0 \pm 0.16	0.84 \pm 0.16	0.66 \pm 0.16	1.0 \pm 0.16
Liver				
MDA ($\mu\text{g}\cdot\text{mg}^{-1}$ protein)	1.51 \pm 0.09	1.35 \pm 0.11	1.49 \pm 0.07	1.37 \pm 0.05
GPx (nkat $\cdot\text{mg}^{-1}$ protein)	27.33 \pm 2.16 ^a	29.34 \pm 2.33 ^b	41.51 \pm 2.50 ^{abc}	21.84 \pm 3.00 ^c
Kidney				
MDA ($\mu\text{g}\cdot\text{mg}^{-1}$ protein)	0.91 \pm 0.11	0.84 \pm 0.07	0.97 \pm 0.11	0.88 \pm 0.06
GPx (nkat $\cdot\text{mg}^{-1}$ protein)	11.50 \pm 1.00 ^a	12.50 \pm 0.83	16.17 \pm 1.33 ^a	13.83 \pm 0.66

Significant differences within a row are indicated by the same superscript letter ($P < 0.05$)

Table 3. Concentration of malondialdehyde (MDA) in plasma, activity of glutathione peroxidase (GPx) in blood, superoxide dismutase (SOD) in red blood cells (RBC), activities of catalase (CAT), alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma-glutamyl transferase (GGT) in plasma and -SH group in blood of broilers fed diets supplemented with different concentrations of *Cinnamomum zeylanicum* oil (means \pm SEM; n = 8 in each group)

	Control	0.10%	0.05%	0.025%
MDA ($\mu\text{mol}\cdot\text{l}^{-1}$)	0.32 \pm 0.04 ^a	0.23 \pm 0.01 ^a	0.24 \pm 0.01	0.25 \pm 0.01
GPx ($\mu\text{kat}\cdot\text{g}^{-1}$ Hb)	2.19 \pm 0.31 ^a	3.64 \pm 0.39 ^a	2.96 \pm 0.21	2.71 \pm 0.31
SOD ($\mu\text{kat}\cdot\text{g}^{-1}$ Hb)	62.44 \pm 3.73	59.82 \pm 4.10	58.16 \pm 4.43	60.74 \pm 4.96
CAT ($\mu\text{kat}\cdot\text{g}^{-1}$ Hb)	2.34 \pm 0.36	2.33 \pm 0.32	2.29 \pm 0.27	2.39 \pm 0.31
ALT ($\mu\text{kat}\cdot\text{l}^{-1}$)	1.53 \pm 0.09 ^{ab}	1.24 \pm 0.04	0.88 \pm 0.21 ^a	0.95 \pm 0.09 ^b
AST ($\mu\text{kat}\cdot\text{l}^{-1}$)	2.81 \pm 0.28	2.84 \pm 0.20	2.60 \pm 0.30	2.61 \pm 0.15
GGT ($\mu\text{kat}\cdot\text{l}^{-1}$)	1.55 \pm 0.08 ^a	1.35 \pm 0.12	1.09 \pm 0.11 ^a	1.15 \pm 0.11
-SH group (mmol $\cdot\text{l}^{-1}$)	0.28 \pm 0.02	0.27 \pm 0.02	0.28 \pm 0.02	0.30 \pm 0.02

Significant differences within a row are indicated by the same superscript letter ($P < 0.05$)

Table 3 shows the results of indicators in the blood and blood plasma. The MDA activity in plasma was significantly lower only in the group supplemented with 0.1% of essential oil compared to the control group. The ALT activity was significantly reduced in groups supplemented with 0.05% and 0.025% of essential oil compared to the control group. Similar reduction was observed in the activity of GGT in the group containing 0.05% of essential oil. No significant effects of the diets on SOD, CAT and AST activities and concentration of -SH-group were found. The phagocytic activity significantly increased in chickens fed diets supplemented with 0.1% of essential oil while the index of phagocytic activity was affected by the diet supplemented with 0.025% of essential oil (Table 4).

Table 4. Blood phagocytic activity and its index in broilers fed diets containing different concentrations of *Cinnamomum zeylanicum* essential oil (mean \pm SEM; n = 8 in each group).

	Control	0.10%	0.05%	0.025%
Phagocytic activity (%) ^a	8.39 \pm 0.53 ^a	17.01 \pm 1.25 ^{abc}	12.58 \pm 1.44 ^b	11.41 \pm 1.04 ^c
Index of phagocytic activity [‡]	0.49 \pm 0.07 ^a	0.37 \pm 0.08	0.37 \pm 0.08	0.18 \pm 0.03 ^a

^aNumber of white cells containing at least three engulfed particles/100 white cells (neutrophils and monocytes);

[‡]Number of engulfed particles per total number of neutrophils and monocytes observed. Significant differences within a row are indicated by the same superscript letter ($P < 0.05$)

Discussion

We found significantly lower lipid peroxidation in plasma and duodenal epithelium of chicks fed the diet supplemented with 0.10% of essential oil. Other diets containing 0.05% and 0.025% of essential oil had no effect on lipid peroxidation. In our experiment cinnamon had no significant effect on the concentration of MDA in the liver and kidney tissue. Another study indicates that *Cinnamomum zeylanicum* essential oil inhibits the hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity in rats, resulting in a lower hepatic cholesterol content, and suppresses lipid peroxidation via the enhancement of hepatic antioxidant enzyme activities (Lee et al. 2003). It has been shown that *Cinnamomum zeylanicum* essential oil is not suitable for the test of MDA, because the main component trans-cinnamaldehyd strongly interacts with the thiobarbituric acid used in the assay, developing yellow colour, which made the experiment impossible to carry out (Baratta et al. 1998). We used the spectrofluorimetric method of Jo and Ahn (1998), where the n-butanol phase is clear and the fluorimetric assay is not based on colour absorbance. It has been reported that lower GPx activity is generally accompanied with an increase of the MDA concentration (Balogh et al. 2004). The digestive tract itself is considered to be a major site of free-radical production in animals and some of them might be delivered via portal blood system into the liver. In our study, ALT was significantly reduced in chickens fed diets supplemented with 0.05% and 0.025% of cinnamon. The AST activity was not affected by diets. It shows that cinnamon has an effect on the liver function. Our results indicate that cinnamon has hepatoprotective effects. SOD plays an important role in protecting cells from damage caused by different reactive oxygen species. SOD converts superoxide to H₂O₂ and oxygen. In turn, H₂O₂ is reduced by catalase and GPx to H₂O and oxygen. Normally, GPx is considered qualitatively more important in maintaining low cellular H₂O₂ levels because it has a much lower Km than catalase (Jones et al. 1981). Our results show that only the GPx activity in blood erythrocytes was affected by *Cinnamomum zeylanicum* essential oil as potential antioxidant. SOD and CAT were not significantly affected in chickens fed diet supplemented with *Cinnamomum zeylanicum* essential oil. It was observed that feeding diets containing different doses of essential oils affected the GPx activity in the blood of mice (Faix et al. 2007). Differences in antioxidant activities of cinnamon might arise from different parts of plant. Cinnamon leaf oils have very high antioxidant activities, whereas cinnamon bark oils have low antioxidant activities (Juliani et al. 2004). Barks of *Cinnamomum* plants are used as spice and herbal medicine, and *Cinnamomum zeylanicum* bark contains dimeric, trimeric, and higher oligomeric proanthocyanidins with doubly linked bis-flavan-3-ol units in the molecule. This class of proanthocyanidins is known to occur widely in common foods (Gu et al. 2004) as well as the singly linked proanthocyanidins. Jayaprakasha et al. (2006) isolated and identified non-volatile constituents and as well as antioxidant activities from cinnamon fruits.

Cinnamomum zeylanicum essential oil showed stimulatory effects on macrophages. Phagocytosis and killing of invading microorganisms by macrophages constitute the body's primary line of defence against infections (Van Furt 1982). Macrophages are an integral

part of the immune system, acting as phagocytic, microbicidal and tumouricidal effector cells. Through interaction with lymphocytes, macrophages play an important role in the initiation and regulation of the immune response (Albercht 1979; Kende 1982). Most of the plants so far reported for immunomodulatory effects have major effects on non-specific immunity, i.e. macrophage functions (Atal et al. 1986; Dhuley 1997).

In conclusion, the present investigation shows that *Cinnamomum zeylanicum* essential oil exhibits significant antioxidant activity in broiler chickens. Thus, it appears that this spice exerts antioxidant protection through its ability to activate the antioxidant enzymes. Findings of the present study establish that *Cinnamomum zeylanicum* essential oil has appreciable immunostimulatory activity.

Vplyv éterického oleja *Cinnamomum zeylanicum* na antioxidačný status broilerov

V pokuse sme sledovali vplyv éterického oleja *Cinnamomum zeylanicum* na antioxidačný status kurčiat. Tridsať dva broilerov plemena Ross 308 sme kŕmili jednou zo štyroch diét doplnenou o éterický olej v koncentráciách 0 %, 0,1 %, 0,05 % a 0,025 % po dobu 38 dní. Vzorky krvi, pečene, obličiek a duodenálneho epitelu sme odobrali na následné sledovanie antioxidačného statusu. Kŕmenie diétou s doplnkom éterického oleja v koncentrácii 0,1 % významne znížilo koncentráciu malondialdehydu (MDA) v plazme a duodenálnej mukóze v porovnaní s kontrolnou skupinou (0 %). Aktivita glutatión peroxidázy (GPx) bola významne vyššia v krvi kurčiat kŕmených diétou s doplnkom éterického oleja v koncentrácii 0,1 %. Diéty obsahujúce éterický olej v koncentráciách 0,05 % a 0,025 % redukovali aktivitu alanin amino transferázy (ALT) v plazme v porovnaní s kontrolnou skupinou. Fagocytárna aktivita v krvi bola významne vyššia u kurčiat kŕmených diétou s prídavkom éterického oleja v koncentrácii 0,1 % a index fagocytárnej aktivity pri 0,025 % koncentrácii v porovnaní s kontrolnou skupinou. Prezentované výsledky ukazujú, že éterický olej *Cinnamomum zeylanicum* preukazuje významný vplyv na antioxidačný status výkrmových kurčiat a môže byť používaný pre antioxidačnú ochranu ako prídavok do krmiva.

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