Effects of Dietary Antibiotic and Cinnamon Oil Supplementation on Antioxidant Enzyme Activities, Cholesterol Levels and Fatty Acid Compositions of Serum and Meat in Broiler Chickens

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Abstract

The aim of the present study was to investigate potential hypocholesterolaemic antioxidant activities of cinnamon oil and antibiotic, and their effects on fatty acid compositions of serum and meat in broilers. A total of 240 Ross-308 chicks, five days old, were divided randomly into four treatment groups composed of 60 chicks each. Experimental groups were: corn-soybean meal basal diet (Control), basal diet supplemented with 10 ppm avilamycin (antibiotic) and 500 or 1000 ppm of cinnamon oil (C500, C1000). Cinnamon oil lowered cholesterol levels of serum ($P < 0.01$), breast and thigh meat ($P < 0.05$) in cinnamon groups compared to control and antibiotic groups. Serum malondialdehyde (MDA, nmol/g protein) level was reduced significantly in C1000 group ($P < 0.05$). Glutathione peroxidase (GSH-Px,μkat/g protein) and catalase (CAT, kat/l) enzyme activities were different among the groups ($P < 0.001$). The higher levels of GSH-Px and CAT were obtained in C1000 group, the lower levels of these indicators were obtained in the antibiotic group. Total saturated fatty acid (SFA) ratio decreased and total unsaturated fatty acid (PUFA) ratio, ω-6 fatty acids increased significantly in serum and thigh meat in cinnamon groups ($P < 0.01$). These results showed that cinnamon oil had hypocholesterolaemic and antioxidant characteristics, and it also improved meat quality.

Broiler, cinnamon oil, antioxidant enzyme activities, cholesterol level, fatty acid composition

The ban on the use of antibiotics as growth promoters has stimulated the search for alternative feed supplements in broiler chicken production. In this view, aromatic plants and essential oils extracted from these plants became interesting due to their antimicrobial (Cabuk et al. 2003), antioxidant (Botsoglou et al. 2002), hypocholesterolaemic (Craig 1999) effects and stimulating effects on animal digestive enzymes (Cabuk et al. 2003). The cholesterol lowering property of essential oil constituents has been ascribed to suppressing of 3-hydroxy-3-methylglutaryl coenzyme A reductase (Elson et al. 1989), the enzyme that is considered to be rate limiting in cholesterol synthesis (Goldstein and Brown 1990). Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (Cheeseman 1993). The destruction can lead to cell death and also to the production of toxic and reactive aldehyde metabolites, known as free radicals. Among these free radicals, malondialdehyde (MDA) is the most important (Paradis et al. 1997). MDA is the main final product of lipid peroxidation and has been often used for determining oxidative damage (Sevanian and McLeod 1997) which is indicated by high levels of MDA. Active principles of spices such as curcumin (turmeric), capsaicin (red chillies), eugenol (cloves), linalool (coriander), piperine (black pepper), zingerone (zinger) and cuminaldehyde (cumin) are reported to inhibit lipid peroxidation (Nagababu and Lakshmaiah 1992; Pulla Reddy and Lokesh 1992; Naidu 1995).

Poultry meat has many desirable nutritional characteristics such as low lipid content and relatively high concentrations of polyunsaturated fatty acids (Igene and Pearson 1979). Recently, the importance of polyunsaturated fatty acids in diet has significantly become more recognized. They are known to play very important roles in human health

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and nutrition (Leung and Foster 1996). Fatty acid composition of diet is an important factor that affects fatty acid composition in the skeletal muscles of broilers that consume the diet (Crespo and Esteve Garcia 2001; Azman et al. 2004; Ertas et al. 2005).

Cinnamon (*Cinnamomum zylenicum*) is commonly used in the food industry because of its special aroma. Additionally, it has strong antibacterial properties, anticandidial, antiulcer, analgesic, antioxidant and hypocholesterolaemic activities (Mastura et al. 1999; Lin et al. 2003). With the idea that they may have a significant effect on antioxidant and fat metabolism in broilers, two different levels of cinnamon oil and antibiotic supplemented in the diet and their effects were searched on cholesterol levels, antioxidant system and fatty acid compositions of serum and chicken meat.

Materials and Methods

Experimental design and diet

The study was carried out using a total of 240 Ross-308 chicks, following local research Ethics Committee approval. The chicks, five days old, were obtained from a local hatchery and divided into four groups of 60 each. Each treatment group was further subdivided into four regular replicates of balanced live weight and gender. A corn-soybean meal-based basal diet was formulated by National Research Council (1994) standards; the ingredients and chemical composition of the diets are presented in Table 1. The treatment groups were the basal diet alone (control) or the basal diet supplemented with 10 ppm antibiotic (Avilamycin, Kartal chem., Turkey); 500 ppm and 1000 ppm essential oil extract from cinnamon (*Cinnamomum zylenicum*) (Ozdrog Co., Hatay, Turkey). Vegetable oil was used as the fat source. Cinnamon oil was dissolved in vegetable oil and then gently added to the standard diet, and the antibiotic was mixed carefully with the standard diet. The diets were prepared freshly each day. The diets were isocaloric and isonitrogenous. Feed and water were given *ad libitum*. At the end of study (35th day), six male chickens with a body weight near the group average were selected in each group and slaughtered by cervical dislocation. Blood samples were collected from the jugular vein during slaughter, allowed to clot and centrifuged for 5 min at 2260 × *g* to separate the sera. The sera samples were stored (-20 °C) until analyzed. For the fatty acid analyses of chicken meat, M. pectoralis profundus of breast and M. gastrocnemius of thigh were obtained and stored (-20 °C).

Table 1. Ingredients and chemical composition of standard diets (%)

Feed ingredients (% of the diet)	5 to 21	22 to 35
Corn	51.41	60.83
Soybean meal (44 CP)	38.99	31.30
Vegetable oil	5.50	4.30
Dicalcium phosphate	2.00	1.50
Ground limestone	1.00	1.20
Salt	0.25	0.25
DL-methionine	0.18	0.07
L-lysine	0.17	0.05
Vitamin premix*	0.25	0.25
Mineral premix**	0.25	0.25
Total	100	100
Nutritional composition (% of the diet)		
Dry matter	90.40	90.10
Crude protein	23.00	20.10
Crude fibre	3.85	4.18
Ash	6.25	6.08
Ether extract	6.35	6.28
Ca	1.01	0.91
P	0.45	0.36
Methionine	0.91	0.72
Lysine	1.29	1.08
ME, kcal/kg	3200	3202

*Vitamin premix supplied per kg; vitamin A 12.000 IU; vitamin D_3 5.000 IU; vitamin E 75 IU; vitamin K_3 3 mg; vitamin B_1 3 mg; vitamin B_2 6 mg; niacin 45mg; calcium d-pantothenate 10 mg; vitamin B_6 7.5 mg; vitamin B_{12} 0.03 mg ; folic acid 1 mg; d-biotin 0.15 mg.

**Mineral premix supplied per kg; Mn 100 mg; Fe 60 mg; Zn 60 mg; Cu 5 mg; Co 0.3 mg; I 1 mg; Se 0.35 mg.

Chemical analysis

Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were analyzed according to the AOAC (2000) procedures and crude fibre was determined by the methods of Crampton and Maynard (1983).

Lipid extraction

Extraction of lipids from tissue specimens was carried out with Hara and Radin (1978) method in which 3:2 (v/v) hexane isopropanol mixture was used. For this aim, 1 g tissue specimen was homogenized in 3:2 (v/v) 10 ml hexane-isopropanol mixture for 30 s. Tissue homogenate was centrifuged in 2260 × *g* for 10 min; supernatant was taken and used for tissue analysis.

Analysis of cholesterol amount with HPLC device

Cholesterol analysis was carried out

according to what Katsanidis and Addis (1999) reported. One section of lipid extraction phase which was divided into two sections was put into tubes with caps and 5% KOH solution was added (KOH solution was prepared in 100% ethanol). After mixing thoroughly, it was kept at 85 ºC for 15 min. The tubes were cooled at room temperature, 5 ml of pure water were added and the fluid was vortexed. After phase separation, upper hexane phase was taken and its solvent was evaporated. Then it was solved with nitrogen flow in acetonitryl/methanol mixture (%50 + %50, v/v) and was taken to autosampler vials, and was prepared for analysis.

For the mobile phase, acetonitryl/methanol (%60 + %40, v/v) mixture was used. The mobile phase flow speed was 1 ml/min. An UV detector was used for the analysis. The wavelength was 202 nm. For the column, Supelcosil LC 18 (15 × 4.6 cm, 5 µm; Sigma, USA) column was used.

Lipid peroxidation

The levels of malondialdehyde (MDA) were measured in serum with the thiobarbituric acid reaction by the method of Placer et al. (1966). The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane. Every sample was assayed in duplicate, and the assay coefficients of variation for MDA were less than 3%.

Catalase (CAT, E.C. 1.11.1.6)

Serum catalase activity was measured as previously described by Goth (1991). Briefly, 0.2 ml of serum samples was incubated in 1.0 ml substrate (65 µmol per ml hydrogen peroxide in 50 mM phosphate buffer, pH 7.0) at 37 ºC for 60 s. The enzymatic reaction was terminated with 1.0 ml of 32.4 mM ammonium molybdate. Hydrogen peroxide was measured at 405 nm against blank containing all the components except the enzyme on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan).

Glutathione peroxidase (GSH-Px, E.C. 1.11.1.9)

The GSH-Px activity was determined according to the method of Lawrence and Burk (1976). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM ethylene diamine tetra acetic acid (EDTA), 1 mM sodium azide (NaN_3), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 IU/ml oxidized glutathione (GSSG)-reductase, 1 mM GSH, and 0.25 mM hydrogen peroxide (H_2O_2). Enzyme source (0.1 ml) was added to 0.8 ml of the above mixture and incubated at 25 °C for 5 min before initiation of the reaction with the addition of 0.1 ml of peroxide solution. The absorbance at 340 nm was recorded for 5 min on a spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value.

Reduced glutathione (GSH)

The GSH content of the serum was measured at 412 nm using the method of Sedlak and Lindsay (1968). The samples were precipitated with 50% trichloroacetic acid and then centrifuged at 1000 × *g* for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris-EDTA buffer (0.2 M; pH 8.9) and 0.1 ml of 0.01 M 5,5'-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min, and then read at 412 nm on the spectrophotometer.

Determination of protein content

The protein content in serum was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Preparation of fatty acid methyl esters

For preparation of methyl esters, lipid extract in the hexane/isopropanol phase was taken into 30 ml experiment tubes. Five ml of 2% methanolic sulphuric acid were added and the mixture was vortexed. This mixture was left to methylate at 50 ºC incubation for 15 h. Then it was cooled at room temperature, 5 ml of 5% sodium chloride were added and mixed. Fatty acid methyl esters that were produced were extracted with 5 ml hexane. Then the hexane phase was taken using a pipette and treated with 5 ml 2% $KHCO_3$. Solvent of methyl ester-containing mixture was evaporated at 45 ºC with nitrogen flow and solved with 1 ml hexane. Then they were taken to closed 2 ml autosampler vials and analyzed in gas chromatography (Christie 1992).

Table 2. Fatty acid composition of finisher diets (%)

Fatty acid	Control	Antibiotic	Cinnamon oil (ppm)	
			500	1000
∑SFA	15.08	13.99	14.16	14.25
∑MUFA	23.66	24.72	24.90	24.31
∑PUFA	60.82	61.29	60.94	61.44
ω-3	4.98	4.92	5.58	5.32
ω-6	55.84	56.37	55.36	55.70

Gas chromatographic analysis of fatty acid methyl esters

Fatty acid methyl esters were analyzed with SHIMADZU GC 17 Ver. Three gas chromatography 25 m long Machery-Nagel (Germany) capillary column with an internal diameter of 0.25 mm and a thickness of PERMABOND 25 micron film was used. During the analysis, column heat was kept at 120-220 °C, injection heat was kept at 240 °C and detector heat was kept at 280 °C. Column heat program was regulated to 220 ºC from 120 ºC, heat increase was set to 5 ºC/min until 200 ºC and to 4 ºC/min from 200 to 220 ºC and kept at 220 ºC for 8 min. As a carrier gas, nitrogen gas and as detector, FID (Flame Ionization Detector) were used. During the analysis, before the analysis of fatty acid methyl esters of the samples, the mixtures were injected to standard fatty acid methyl esters and residence times of each fatty acid were determined. After this treatment, necessary program analysis was made and fatty acid methyl esters mixtures were analyzed.

Statistical analysis

Data were subjected to analysis of variance, significant differences were further subjected to Duncan's multiple range test (SPSS 1993). The results were considered as significant when *P* values were lower than 0.05 and 0.01.

Table 3. The effects of dietary cinnamon oil and antibiotic on total cholesterol levels of broilers up to the age of 35 days (mean ± SEM) (n = 6)

	Control	Antibiotic	Cinnamon oil (ppm)		
			500	1000	*P*
Serum (mmol/L)	3.83 ± 0.11[a]	3.75 ± 0.10[a]	3.12 ± 0.17[b]	3.26 ± 0.11[b]	**
Thigh (mg/100g)	73.20 ± 3.01[a]	71.32 ± 2.98[a]	63.45 ± 2.85[b]	64.12 ± 2.72[b]	*
Breast (mg/100g)	69.17 ± 2.56[a]	69.83 ± 2.59[a]	58.33 ± 2.73[b]	61.50 ± 2.88[b]	*

*: $P < 0.05$, **: $P < 0.01$ [a,b]: Mean values with different superscripts within a row differ significantly

Results and Discussion

The effects of dietary cinnamon oil and antibiotic on total cholesterol levels of broilers are given in Table 3. Total cholesterol of the serum ($P < 0.01$), thigh and breast meat ($P < 0.05$) were found to be lower in both cinnamon groups of the present study. This may be related with cinnamon oil added to the diet and its inhibition mechanism on HMG-CoA reductase activity. Two key enzymes involved in regulating cholesterol metabolism are HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, and ACAT, the cholesterol-esterifying enzyme in tissue. The inhibition of HMG-CoA reductase decreases cholesterol synthesis and its inhibitors are very effective in lowering plasma cholesterol in most animal species, including humans (Alberts 1988). Cinnamic acid (0.02%, w/w) and its synthetic derivatives (HPP304, HPP305) significantly inhibit hepatic HMG-CoA reductase activity and decrease serum total cholesterol level (Lee et al. 2001; Lee et al. 2007). When the effects of essential oils on hypocholesterolemic properties were taken into consideration, the results of the present study were in agreement with the reports of the previous studies (Elson et al. 1989; Yu et al. 1994; Case et al. 1995). Unlike these findings, Lee et al. (2003) failed to show any hypocholesterolemic effects of the active items as thymol, cinnamaldehyde and a commercial preparation of essential oil components (CRINA® Poultry).

According to Table 4, cinnamon oil (1000 ppm) reduced MDA level ($P < 0.05$) and increased GSH-Px and CAT activities ($P < 0.001$). These effects are due to the antioxidant property of cinnamon oil (Lin et al. 2003). The protective role of essential oils may result from its antioxidative defense mechanism through the induction of antioxidant enzyme activities (Hsu and Liu 2004). Choiem Hwang (2005) reported that the intake of medicinal plants in rats results in an increase in antioxidant enzyme activity and a decrease in MDA. Phenolic compounds of essential oils increased the activity of CAT, which in turn detoxifies hydrogen peroxide and converts lipid hydroperoxides to nontoxic substances (Fki et al. 2005). In addition, GSH-Px and CAT activity were found to be lower in the

Table 4. The effects of dietary cinnamon oil and antibiotic on lipid peroxidation and antioxidant enzyme activities of broilers up to the age of 35 days (mean ± SEM) (n = 6)

	Control	Antibiotic	Cinnamon oil (ppm)		P
			500	1000	
MDA (nmol/g protein)	1.38 ± 0.13a	1.38 ± 0.10a	1.36 ± 0.12a	1.18 ± 0.12b	*
GSH (µmol/g protein)	0.09 ± 0.00	0.09 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	NS
GSH-Px (µkat/g protein)	0.14 ± 0.00b	0.13 ± 0.00c	0.15 ± 0.00b	0.17 ± 0.00a	***
CAT (kat/l)	37.45 ± 3.02b	30.52 ± 2.08c	42.69 ± 2.95b	59.06 ± 4.36a	***

NS: $P > 0.05$, *: $P < 0.05$, ***: $P < 0.001$, $^{a\text{-}c}$: Mean values with different superscripts within a row differ significantly.

antibiotic group compared with the other cinnamon groups and control of the present study ($P < 0.001$). Antibiotics produce different effects on CAT and GSH-Px activities in the organism (Johnson et al. 2000). Sukoyan et al. (2005) reported that penicillin, aminoglycosides and cephalosporins decreased enzyme activity of the glutathione-associated antioxidant system, after treatment with antibiotics glutathione peroxidase activity decreased, while catalase activity remained unchanged. In the present study catalase activity also decreased significantly differently than reported by Sukoyan et al. (2005).

The effects of dietary cinnamon oil and antibiotic on fatty acid composition of serum, thigh and breast meat are given in Tables 5, 6 and 7, respectively. Total SFA ratio of the serum and thigh meat lowered and total PUFA, ω-6 fatty acids ratios significantly increased in cinnamon oil groups ($P < 0.05$). Changes in body fat deposition between broilers fed different dietary fatty acid profiles may be related to different rates of lipid synthesis or lipid oxidation (Crespo and Esteve Garcia 2001). Chithra and Leelamma (1999) reported that *Coriander sativum* decreased lipid uptake and enhanced lipid breakdown, resulting in lipolytic effects. This action on lipid metabolism could explain the reduction of SFA contents in meat. On the other hand, enhancement of unsaturated fatty acids in meat lipids would result from diminution of fatty acid oxidation in tissue. Previous studies reported that essential oils, especially cinnamon, had an antioxidant property (Yu et al. 1994; Case et al. 1995; Lee et al. 2001; Lee et al. 2007). This antioxidant property of cinnamon was supported in the present study. It was thought that the antioxidant property of cinnamon blocked lipid peroxidation of tissue lipids, especially polyunsaturated fatty acids. For this reason, polyunsaturated fatty acids of serum and thigh meat were found as significantly high in this study.

Table 5. Fatty acid composition of serum lipids in broilers fed with cinnamon and antibiotic supplemented diets with basal diet (mean ± SEM) (n = 6)

Fatty acid (%)	Control	Antibiotic	Cinnamon oil (ppm)		P
			500	1000	
∑SFA	41.78 ± 4.56^{a}	38.23 ± 0.78^{ab}	34.51 ± 0.46^{ab}	33.77 ± 1.07^{b}	*
∑MUFA	18.79 ± 1.43	21.25 ± 0.65	20.95 ± 1.13	21.40 ± 1.88	NS
∑PUFA	39.43 ± 0.53^{b}	40.52 ± 0.70^{b}	44.54 ± 1.00^{a}	44.83 ± 1.47^{a}	**
ω-3	1.28 ± 0.26	0.96 ± 0.07	1.15 ± 0.21	1.32 ± 0.28	NS
ω-6	38.15 ± 0.53^{b}	39.56 ± 0.70^{b}	43.39 ± 1.00^{a}	43.51 ± 1.47^{a}	***

NS: $P > 0.05$, *: $P < 0.05$, ** : $P < 0.01$, *** : $P < 0.001$, $^{a\text{-}c}$: Mean values with different superscripts within a row differ significantly.

Table 6. Fatty acid composition of thigh meat lipids in broiler fed with cinnamon and antibiotic supplemented diets with basal diet (mean ± SEM) (n = 6)

Fatty acid (%)	Control	Antibiotic	Cinnamon oil (ppm)		P
			500	1000	
∑SFA	33.02 ± 0.65a	30.47 ± 0.55b	26.91 ± 0.48c	27.59 ± 0.53c	**
∑MUFA	27.62 ± 1.23	31.15 ± 1.65	30.47 ± 1.20	30.57 ± 1.49	NS
∑PUFA	39.36 ± 1.30bc	38.38 ± 1.08c	42.62 ± 0.64a	41.84 ± 0.83ab	*
ω-3	2.36 ± 0.14	2.51 ± 0.13	2.84 ± 0.14	2.72 ± 0.24	NS
ω-6	34.17 ± 1.00b	33.50 ± 0.75b	37.75 ± 0.65a	37.04 ± 0.52a	**

NS: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$ $^{a\text{-}c}$: Mean values with different superscripts within a row differ significantly.

Table 7. Fatty acid composition of breast meat lipids in broiler fed with cinnamon and antibiotic supplemented diets with basal diet (mean ± SEM) (n = 6)

Fatty acid (%)	Control	Antibiotic	Cinnamon oil (ppm)		P
			500	1000	
∑SFA	33.97 ± 1.82	32.10 ± 0.41	32.17 ± 0.50	32.00 ± 0.49	NS
∑MUFA	24.70 ± 0.95	25.31 ± 0.21	25.72 ± 1.10	25.05 ± 0.57	NS
∑PUFA	41.33 ± 3.19	42.59 ± 0.49	42.11 ± 1.01	42.95 ± 1.12	NS
ω-3	3.45 ± 0.23	3.50 ± 0.14	3.40 ± 0.40	3.87 ± 0.18	NS
ω-6	35.78 ± 1.82	36.60 ± 0.52	36.60 ± 1.08	36.65 ± 0.77	NS

NS: $P > 0.05$

In conclusion, this study showed that supplementing different concentrations of cinnamon oil in diet (especially 1000 ppm) decreased cholesterol levels of serum and chicken meat. They had positive effects on antioxidant metabolism, besides increased the antioxidant enzyme activity and decreased the serum MDA level. On the basis of these findings, we suggest that cinnamon oil may play an important role as an endogenous antioxidant and could also be applicable as a protective agent against tissue damage. Because of the hypolipidemic and antioxidative properties of cinnamon oil in diets, polyunsaturated fatty acid ratios may increase in serum and meat lipids. In this way, dietary cinnamon supplementation would improve the nutritional quality of chicken meat.

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