The effect of green tea extract (*Camellia sinensis*) supplementation on blood profiles and lipid oxidation in broilers fed high pufa¹

Isti Astuti, Supadmo,† Sugeng Riyanto,‡ and Supriyadi,§

*Faculty of Agriculture, Sebelas Maret University, Solo 62-57731,Indonesia; †Faculty of Animal Science, Gadjah Mada University, Yogyakarta 62-55281, Indonesia; ‡Faculty of Pharmacy, Gadjah Mada University, Yogyakarta 62-55281, Indonesia; and §Faculty of Agriculture Technology, Gadjah Mada University, Yogyakarta 62-55281, Indonesia

ABSTRACT: The objective of the present study was to investigate the effect of green tea extract (GTE) supplementation on blood profiles and lipid oxidation of broiler chicken fed with high polyunsaturated fatty acid. A total 80 day-old-chick male broilers (Lohmann) were randomly allocated into 5 groups, consisting 4 replicates of 4 bird each. Birds were fed with a basal diet that contain 2% fish oil + 2% coconut palm oil (T0, control); T1 (basal diet + 100 mg GTE); T2 (basal diet + 200 mg GTE); T3 (basal diet + 300 mg GTE) and T4 (basal diet + 200 mg vitamine E). Supplemented GTE decused blood serum triglyceride and cholesterol (P<0.05), while malonaldialdehyde (MDA) levels in breast and liver were significantly reduced (P<0.05) by GTE feeding. The result of the study indicated GTE could act as antioxidant in broiler's fed with high PUFA (polyunsaturated fatty acid).

Key words: green tea extract, blood profiles, lipid oxidation, broiler

INTRODUCTION

Nutritional studies has shown that body fat content in poultry can be manipulated by altering dietary polyunsaturated fatty acids (PUFA), specially by the inclusion in the diet of long-chain PUFA. Reported benefits of PUFA on human health have increased the interest in animal products with high amount of these acids (Farrel, 1998; Ferrer *et al.*, 2001). However, increasing the degree of PUFA in chicken meat enriched number of double bond which increases the susceptability of meat to oxidation (Maraschiello *et al.*, 1999; Grau *et al.*, 2001; Fellenberg and Speisky, 2006). Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life. One such product is malonaldialdehyde (MDA), which considered as an index of oxidative rancidity (Cortinas *et al.*, 2005).

Morrisey *et.al.*, (1997) recommended the use of dietary antioxidant to reduce lipid peroxidation in the feed and animal, and to preserve product quality. Recent research on antioxidants has focused on naturally to eliminate consumers concern about the safety an toxicity of the synthetic counterpark. Extracts of herbs and spices have been studied for their potential to preserve food. Rababah *et al.*, (2006) found that green tea extract had high antioxidant activities to prevent lipid peroxidation. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols.Polyphenol moleculs present in plant can act like antioxidant. The antioxidant action was shown to be dependent on the ability of their constituent phenolic compounds to scavenge free radical and to chelate metals. On the other hand, Samman *et al.* (2001) reported that presence of the phenolic compound resulted in decreased nonheme-iron absorption.

The aim of this study was to determine the effect of supplementation of green tea extract on blood profile and lipid oxidation in broiler fed high PUFA.

¹ I am indebted to the students at Gadjah Mada University, Yogyakarta, Indonesia. Financial support for the study was obtained from Directorate General of Higher Education, Ministry of Education Republic Indonesia. My specials thanks are owing to Dr. Supadmo, MS, Professor. Dr. Sugeng Riyanto, MS, Apt. and Dr. Supriyadi, M.Sc. from Gadjah Mada University for their kind help in manuscript preparation.

MATERIALS AND METHODS

Animal and Diets

An extract of green tea (GTE) was prepared by maceration . Briefly, 10 kg green tea powdered was extracted overnight with 25 L of ethanol 75% by maceration. Extract was filtered with Whatmann paper. This was repeated 2 to 3 times and filtrate was consentrated by rotary evaporator and then the concentrate was made as powder form. Experiment was carried out with 80 one-day-old Lohmann male broiler chickens from local hatchery (Multibreeder Adirama, Indonesia). There were weighed individually, labeled with wing-ring, and allocated to 5 treatments with 4 replicated cages of 4 birds each and reared for 35 d. Dietary treatments were formulated following NRC (1998) (Table 1), consist of corn-soybean meal with 2% fish oil+2% coconut palm oil as a basal diet (T0), a basal diet + 100 mg GTE/kg diet (T1), a basal diet + 200 mg GTE/ kg diet (T2), a basal diet + 300 mg GTE/ kg diet (T3) and a basal diet + 200 mg vit E/ kg diet (T4). At the end of feeding trial, before slaughtering, about 5 mL the sample of blood was collected from wing vein from one bird in each pen (4 chicks per treatment). The blood samples were centrifuged at 5000 rpm and then serum was collected for analysis. The amount of tryglicerida, cholesterol, low-density lipoprotein (LDL) and high density lipoprotein (HDL) were determined by using commercial kit. After slaughtering, breast meat and liver were removed and frozen at -30⁰ C until analysis.

Ingridients	Starter, %	Grower, %	
Yellow maize	57	60	
Soybean meal	31	28	
Poultry meat meal	5	5	
Fish oil	2	2	
Coconut Palm Oil	2	2	
Dicalsiumphosphate	1.40	1.30	
Limestone	1.0	1	
Salt	0.2	0.2	
Vitamin premix	0.2	0.2	
DL methionine	0.2	0.2	
Calculated nutrient content			
Metabolizable energy, kcal/kg	3060	3091	
Crude protein	21.19	20	
Phosphor av.	0.43	0.4	
Calcium	1.00	0.9	

Tabel 1. Nutrient composition of the basal diets

Blood Parameter Assay

Blood samples were centrifuged (at 2,000xg for 10 min) and serum was separated and then stored at -20^oC until assayed for measuring blood parameter (cholesterol, triglycerides, LDL and HDL) using appropriate laboratory kits.

Lipid Oxidation Assay

The analyses was performed by using the method of Maraschiello *et.al.* (2000). Briefly ultrapure water (20 mL) was added to 1,5 g of broiler meat. Sample homogenization was carried out at 13500 rpm for 10 s. Five mL cold 25% trichloroacetic acid (TCA) was added to the homogenate followed by gentle stirring at 4° C for 15 min. The extract was obtained by centrifugation at 13000x g for 15 min at 4° C and 3,5 mL of the supernatant was transferred to a test tube and 1,5 mL of 0,6% aquaeous thiobarbituric acid (TBA) was added. The screw-capped test tube was incubated fo 30 min in a water bath at 70° C. The tube was cooled and the hiobarbituric acid reactive substances (TBARS) were recorded at 532 nm by Shimadzu spectrophotometer againt a blank consisting of 2,5 mL ultrapure

H2O, 1 mL 25% aquaeous TCA and 1,5 mL 0,6% TBA. Calibration curves were prepared using malonaldehvde (MDA) standart working solution.

Analysis of Data

The data were expressed as a mean \pm standard deviation (SD). For all parameters, statistical examination of treatment effect was determined by ANOVA and the differences among the mean values were test by Duncan's multiple- range test. Statements of statistical significance are based on *P*<0.05.

RESULTS AND DISCUSSION

The mean of constituent in serum as triglyceride, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) are presented in the Table 2.

Table 2. The effect of green tea extract supplementation on blood serum profile of broiler chickens at 35 d of age

	Supplement level (mg/kg diet)					
Parameter	0	100 GTE	200 GTE	300 GTE	200 Vit E	
Triglycerida, mg/dL	$100,57 \pm 10,26^{a}$	$89,44 \pm 2,46^{a}$	$88,56 \pm 4,40^{ab}$	$85,32 \pm 4,16^{bc}$	$84,33 \pm 5,36^{\circ}$	
Cholesterol, mg/dL	141,18 ±2,41 ^a	136,76±4,83ª	132,55 ±7,93 ^{ab}	127,77 ± 6,41 ^{bc}	127,49±6,11°	
LDL-cholesterol (mg/dL	51,65 ±2,74	50,50 ±3,79	49,99 ±3,83	47,73 ±2,86	47,74 ±2,85	
HDL cholesterol, mg/dI	L70,67 ±2,77	$68,37 \pm 2,36$	$64,85 \pm 7,55$	62,97 ±6,69	$62,89 \pm 6,28$	
<u>VLDL</u> , mg/dL a,b Means with unlike su	18,84 ±1,17	17,88 ±0,49	17,71 ±0,88	17,06 ±0,83	16,86±1,16	

Means with unlike superscript within same row were different (P < 0.05)

Statistical analysis shows that supplementation green tea extract significantly decrease serum triglycerida and cholesterol but insignificantly on LDL, HDL and VLDL. However, the maximum decrease was observed on the supplementation 300 mg GTE/ kg diet and similary with 200 mg vit. E / kg diet. Similar result have been shown in poultry fed green tea powder and vitamin E (Biswas and Wakita, 2001; Eid et al., 2003). Green tea containing high catechin may have an inhibitory effect on intestinal absorbtion of lipid (Hsu et.al., 2006). The lowering serum cholesterol may also lead by the supressing the posttranscriptional action of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway of endogenous cholesterol synthesis by liver (Rahmatnejad et al., 2009).

Table 3. The effect of green tea extract supplementation on lipid oxidation breast and liver of broiler chickens at 35 d of age

	Supplement level (mg / kg diet)					
Parameter	0	100 GTE	200 GTE	300 GTE	200 vit. E	
Breast meat	2,64	2,34	2,14	1,93	1,99	
(mg	±	±	±	±	±	
malonaldehyde/g	0,38 ^a	0,134 ^{ab}	0,373 ^b	0,156 ^b	0,77 ^b	
meat						
Liver	3,05	2,95	2,85	2,70	2,61	
(mg	±	±	±	±	±	
malonaldehyde/g	0.122 ^a	0.198 ^a	0.08^{ab}	0.092^{bc}	0.051 ^c	
meat						

^{a,b M} eans with unlike superscript within same row were different (P < 0.05).

Supplementary effect of green tea extract on lipid oxidation was presented in Table 3. Malonaldehyde is widely used marker to estimate the degree of oxidation in various biological materials. The breast meat and liver of chicken fed GTE showed significantly lowered malonaldehyde compared with the control. However, the maximum decrease was found in supplementation 300 mg GTE/ kg diet and similary with 200 mg vit. E / kg diet. This result agree with previus outhor that green tea containing high polyphenol, mainly catechin, that have antioxidant activities so they can improve the quality of meat. Phenolic coumpounds present in natural plants react with lipid and hydroxyl radical and convert them into stable produc. Tea catechins effectively reduced inhibited lipid oxidation in raw and cook beef meat, raw breast meat of broiler (Tang et al., 2000; Biswas and Wakita, 2001)

CONCLUSIONS

In conclusion, dietary supplementation of green tea extract (GTE) could decrease triglycerida and cholesterol levels in chick serum and prevent lipid oxidation of breast meat and liver of chick. Our results suggest that dietary supplementation green tea extract at 300 mg/kg diet of broilers is favorable to improve quality broiler production.

LITERATURE CITED

- Biswas, M.A.H. and Wakita, M., 2001, Effect of dietary Japanese green tea powder supplementation on feed utilization and carcass profile in broilers. J Poult Sci 38 (1): 50-57
- Cortinas, L., A. Barroeta, C. Villaverde, J. Galobart, F. Guardiola and M.D. Baucells. 2005. Influence of the dietary polyunsaturation level on chicken meat quality: Lipid oxidation. Poultry Sci. 84: 48-55.
- Eid, Y., A. Ohtsuka and K. Hayashi. 2003. Tea polyphenols reduce glucocorticoid induced growth inhibition and oxidation stress in broiler chickens. British Poultry Science 44: 127-132
- Hsu,T.F., A. Kusumoto, K.Abe, K. Hosoda, Y. Kiso, M-F Wang and S Yamamoto. 2006. Plyphenol-enriched oolong tea increases fecal lipid excretion. Europan Journal of Clinical Nutrition 60: 1330-1336.
- Farrel, D.J., 1993. Manipulating The Composition of The Eggs to Improve Human Health. RPAN Seminar A New Conceptin Poultry Feed Technology, Jakara. 16 September 1993.
- Fellenberg, M.A. and H. Speisky. 2006. Antioxidants : Their effect on broiler oxidative stress and its meat oxidative stability. World Poultry Science Journal. 62 : 53-70
- Ferrer,S.L.,M.D.Baucells,A.C.Barroeta and M.A.Grashorn. 2001. N-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality; Fish oil. Poultry Sci 80: 741-752
- Grau,A., F.Guardiola, S.Grimpa, A.C.Barroeta and R.Codony. 2001. Oxidative stability of dark chicken meat throung frozen storage: Influence of dietary fat and α-tocopherol and ascorbic acid suplementation. Poult. Sci., 80: 1630-1642
- Marascheillo,C.,C.Sarraga,E.Esteve-gracia and J.A.GraciaRegueiro. 2000. Dietary iron and copper removal does not improve cholesterol and lipid oxidative stability of raw and cooked broiler meat. J. Food Sci. 65 (2) :211 214
- Morrisey, P.A., Brandon, S., Buckley , D.J., sheehy, P.J.A. and Friegg, M. 1997. Tissue content of alfa tocopheryl acetat supplement for various periods pre-slauhgter. British Poultry Science 38 : 84-88
- Rababah, T., N.S. Hettyarachchy, R.Horax, M.J.Cho, B.Davis and J.Dickson. 2006. Thiobarbuturic acid reactive substances and volatile compounds in chicken breast meat infused with plant extracts and subjected to electron beam irradiation. Poultry Sci. 85:1107-1113.
- Rahmatnejad, E., M.Bojarpour, Kh.Mirzadeh, M.Chaji and T. Mohammadabadi. 2009. The effect of different levels of dried tomato pomace on broilers chicken hematological indices. J. Animal and Veterinary Advances 8 (10) : 1989-1992.
- Samman, S., B. Sandstrom, M. B. Toft, K. Bukhave, M. Jensen, S.S. Sorensen and M. Hansen. 2001. Green tea or rosemary extract added to foods reduces nonhem-iron absorption. Am. J. Clin. Nutr. 73: 607-612