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Full Length Research Paper

Dietary supplementation of green tea by-products on growth performance, meat quality, blood parameters and immunity in finishing pigs

Md. Elias Hossain, Seok Young Ko and Chul Ju Yang*

Department of Animal Science and Technology, Sunchon National University, Suncheon, Jeonnam 540-742, Korea.

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Green tea has a long-standing reputation for its health-promoting properties. It has a useful content of amino acids, proteins, vitamins, tannins and polyphenols, such as epicatechin, epigallocatechin, epigallocatechin gallate and gallocatechin. This study evaluated the potential use of green tea by-products in finishing pig diet. A total of 100 finishing pigs were assigned to 5 dietary treatments with 4 replications for 6 weeks in a completely randomized design. The treatments were as follows: control (basal diet), antibiotic (basal diet with 0.003% chlortetracycline), and basal diet with 0.5, 1 or 2% green tea by-products (GTB). In this experiment, a poor weight gain and feed conversion ratio was observed for the GTB-2% group when compared with the antibiotic group. Both crude protein and crude fat contents of the meat were inversely proportional to each other in the GTB groups and 0.5 to 1% level differed with the antibiotic group. The slaughter weight and shear value were higher in GTB-1 and 0.5%, group respectively, while a lower heating loss and higher tenderness were observed in the GTB-2% group. Supplementation of the pig diet with GTB reduced the thiobarbituric acid reactive substances values of the meat, and increased white blood cells (WBC) and red blood cells (RBC) content compare to others. Although, spleen weight was decreased in the GTB-1 and 2% groups, spleen cells growth and, IL-6 and TNF-α production were improved by the addition of GTB to the feed. These combined results indicated that 0.5 to 1% GTB hold great promise to use as feed additive for finishing pigs.

Key words: Green tea by-products, growth, meat, blood, immunity, pigs.

INTRODUCTION

Recent consumer scares over animal production practices have renewed interest in using alternative ingredients to antibiotics, particularly those from plants, which are perceived as ‘natural’ and ‘safe’ by consumers. However, plant extracts are in many commercial preparations currently used in animal production, and produce antimicrobial (Jamroz et al., 2003), antioxidant (Cross et al., 2007), antitoxin effects (Platel and Srinivasan, 2000); and are able to improve digestibility (Rao et al., 2003), stimulate enzyme activity (Platel et al., 2002) and immune functions (Ko and Yang, 2008). Consumption of ready-made tea drinks such as green tea (*Camellia sinensis*) has increased markedly in ecent years in Southeast and East Asia. World green tea production was 0.968 million metric ton (MMT) in 2006 and the projected production in 2017 is 1.571 MMT (Hicks, 2009). Consequently, the amount of tea by-products, which are the residues of tea drink manufacturing at beverage companies, has been increasing.

In fact, in 2003, more than 200,000 tons of used tea leaves were produced from all kinds of tea, of which approximately 90,000 tons were from green tea and 40,000 tons from black tea (Kondo et al., 2005). Used green tea leaves from factories are disposed of as compost, dumped into landfills or burned, which causes both an economical and environmental problem. It is important to reuse tea wastes in livestock production to reduce the use of artificial additives and environmental

*Corresponding author. E-mail: yangcj@scnu.kr. Tel: 82-61-750-3235. Fax: 82-61-750-3239.

effect. Green tea has over 200 bioactive compounds and contains over 300 different substances (Labdar, 2010). Numerous *in vitro* and *in vivo* studies on green tea preparations have demonstrated the antimutagenic, anticarcinogenic and antioxidant properties of the polyphenolic compounds, which are mainly composed of four types of catechin: epicatechin, epigallocatechin, epigallocatechin gallate and gallocatechin (Yamamoto et al., 1997). The predominant green tea component has been shown to improve body weight gain and feed efficiency in pig (Ko et al., 2008), cattle (Sarker et al., 2010) and broiler (Biswas and Wakita, 2001).

In addition, this component maintains microflora balance and displays antimicrobial effects against pathogenic bacteria (Hara-Kudo et al., 2005). It has been shown that green tea polyphenols have strong antioxidation properties (Nishida et al., 2006). It reduces thiobarbituric acid reactive substances (TBARS) values and maintain oxodative stability of pig meat (Ko et al., 2008), broiler meat (Yang et al., 2003) and egg yolk (Uuganbayar et al., 2005). Yang et al. (2003) reported that cholesterol levels were decreased and fatty acids of plasma and meat were improved, when the animals were fed different levels of green tea by-products (GTB). Supplementation of GTB and green tea with probiotics has no negative effect on the blood components of beef cattle and calves (Lee, 2005; Sarker et al., 2010). Ko and Yang (2008) found that adding 0.5% GTB in the finishing diet produced positive effects on the humoral and cell-mediated immunity of pigs.

In the present study, we evaluated the activity of GTB to determine the specific effects of this component on growth performance, carcass characteristics, meat quality, blood parameters and immune response in finishing pigs and also to determine the effective level in finishing pig diet.

MATERIALS AND METHODS

Animals, diets and experimental design

A total of 100 crossbreed (Landrace × Large White) castrated male finishing pigs with an average body weight of 77 ± 0.4 kg were assigned to 5 dietary treatments in a completely randomized design. Each treatment had 4 replications with 5 pigs per replication. The pigs were reared in the experimental farm at Sunchon National University following the guidelines for the care and use of animals in research (Korean Ministry for Food, Agriculture, Forestry and Fisheries, 2008). All animals were fed experimental diets for 6 weeks.

The five dietary treatments were as follows: control (basal diet), antibiotic (basal diet with 0.003% chlortetracycline) and basal diet with 0.5, 1 and 2% GTB. Used green tea, residues from green tea drink manufacturing were collected from the green tea experimental station (Boseong, South Korea), dried, grounded and used for experiment. GTB was analyzed and the main constituents of were found as moisture 10.15%, crude protein 20.16%, crude fat 3.08%, crude fiber 19.20%, crude ash 5.22% and catechins 7.17%. All diets were formulated to meet or exceed nutrient requirements of finishing pigs (NRC, 1998). The formula and chemical composition of the basal diet used in this experiment are provided in Table 1.

Measurements and analysis

Growth performance, carcass characteristics and oxidative stability of meat

Body weights were measured on a biweekly basis from the beginning to the end of the experiment. Feed intake was determined by measuring feed residue on a biweekly basis from the start of the experiment. Feed conversion ratio (FCR) was obtained by dividing the feed intake by body weight gain. At the end of the experiment, pigs were transported to the slaughter house (Naju, Korea). Carcass weight and back fat thickness were measured, and carcass grade were determined according to Animal Products Grade System (Korea Institute for Animal Products Quality Evaluation, 2011). All the pork carcasses in South Korea are graded both in quality and conformation terms. The quality of pork carcasses is graded 1^+, 1 and 2 based on the marbling, lean color and conditions of belly streaks. The conformation terms of pork carcasses is graded A, B and C by assessing carcass weight, back fat thickness, balance, muscle, fat condition and so on. Carcass grades in this study were expressed as 3 (extremely good), 2 (good) and 1 (bad) in A, B and C grades of conformation terms. The thiobarbituric acid reactive substances (TBARS) value of pork was assayed using the methods described by Vernon et al. (1970) with slight modifications. Four gram of loin meat mixture was blended at full speed for 1.5 min with 10 ml of solution containing 20% trichloroacetic acid in 2 M phosphoric acid. The resulting sediment was transferred to 100 ml volumetric flask containing 8 ml distilled water and diluted by shaking and homogenized. The mixture was filtrated through Whatman No. 1 filter paper. Then 5 ml filtrate was transferred to a 20 ml test tube and 5 ml of 2-thiobarbituric acid (0.005 M in distilled water) was added. The solution was shaken in a water bath at 80℃ for 30 min. After cooling, the color development was measured at 530 nm using a VIS-Spectrophotometer (Model $20D^+$, Milton Roy, PA, USA). Thiobarbituric acid reactive substances (TBARS) values were expressed as micromole of malondialdehyde per hundred gram of meat.

Meat composition, quality and sensory evaluation

Moisture, crude protein, crude fat, and ash percentage of the meat samples were analyzed according to Association of Analytical Communities (AOAC, 2000) methods. Color values on a freshly cut surface (3 cm thick slice) of loin meat were measured using a CR-301 Chroma Meter (Minolta Co., Osaka, Japan) for International Commission on Illumination (CIE) standard of lightness (*L*), redness (*a*) and yellowness (*b*). Muscle pH values were determined using a pH meter (ATI 370, Orion Research Inc, MA, USA). The meat samples were broiled in a water bath at a temperature of 70℃ for 30 min, surface dried, and weighed. Cooking loss was determined by expressing the cooked sample (B) weight as a percentage of the precooked sample (A) weight. Cooking loss (%) = [(A−B)/A] × 100. Water holding capacity was determined according to the procedure described by Wardlaw et al. (1973). Shear force was measured using the Instron Universal Testing Machine (Model 4465, Instron Corp., Norwood, MA, USA), which was equipped with a Warner-Bratzler shear device. From each cooked meat sample, a 0.5 × 4.0 cm (approximately 2.0 cm^2) cross section was cut for shear force measurements. The meat samples were placed at right angles to the blade. The crosshead speed was 100 mm/min and the full scale load was 50 kg. Sensor evaluation of pork meat was organoleptically evaluated by ten trained judges from the

Table 1. Ingredients and chemical composition of the basal diet.

Ingredients (%, as-fed basis)	Amount
Corn	44.65
Wheat	25.00
Wheat bran	4.00
Soybean meal	13.50
Lupin seeds	3.00
Limestone	0.77
Tricalcium phosphate	1.10
Salt	0.25
Vitamin-mineral premix[1]	0.56
Animal fat	2.50
Molasses	4.50
L-lysine	0.17
Chemical composition (% dry matter)	
Crude protein	15.00
Calcium	0.78
Available phosphorus	0.55
Lysine	0.80
Methionine	0.27
Metabolisable energy (kcal/kg)	3160

[1]Provided the following nutrients per kg of diet: vitamin A, 6000IU; vitamin D3, 800IU; vitamin E, 20IU; vitamin K3, 2 mg; thiamin, 2 mg; riboflavin, 4 mg; vitamin B6, 2 mg; vitamin B12, 1 mg; pantothenic acid, 11 mg; niacin, 10 mg; biotin, 0.02 mg; Cu, 21 mg; Fe, 100 mg; Zn, 60 mg; Mn, 90 mg; I, 1.0 mg; Co, 0.3 mg; Se, 0.3 mg.

Department of Animal Science and Technology at Sunchon National University on the three point hedonic scale for juiciness, tenderness and flavor.

Biochemical and hematological parameters of blood

Blood samples were collected from the jugular vein of the pigs by venipuncture on the last experimental day, 3 h after feeding. To analyze the biochemical composition of plasma, blood samples were separated by centrifuging for 20 min at 1500 (× g) and were total protein, cholesterol, glucose, albumin, globulin, albumin/globulin (A/G) and blood urea nitrogen (BUN) concentrations were measured using a blood analyzer (COBAS MIRA: Roche, Germany). Number of white blood cell (WBC, $10^3/mm^3$), red blood cell (RBC, $10^6/mm^3$) and hemoglobin concentration (g/dl) were determined using a hematological analyzer (XE-2100, Automated Hematology Analyzer, Sysmex America, Inc.) within 3 h after blood sampling.

Spleen cells culture and proliferation

The immune response of pig spleen cells was analyzed at the end of the experiment. At one third areas of the spleens of pigs, a 1 cm^3 tissue sample was extracted and split into single cell on bovine serum medium (RPMI-1640). Next, NycoPrep™ 1.077A was used to remove the dead cells and red blood cells. Spleen cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM l-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin, 1% (v/v) nonessential amino acids, and 0.05 mM 2-mercaptoethanol (Gibco Paisley, UK) at 37℃, 5% CO_2 condition. Viable cells were counted in a hemocytometer by trypan blue exclusion.Triplicate cultures were performed in 96-well flat-bottomed tissue culture plates (Costar, Cambridge, MA, USA) in a final volume of 200 μl per well containing 5×10^5 cells in the presence of different concentrations of LPS (lipopolysaccharide; 1.0, 3.0 and 10 μg/ml–Sigma-Aldrich, Saint Louis, MO, USA) or Con A (concanavalin A; 0.1, 0.3 and 1.0 μg/ml–Sigma-Aldrich, Saint Louis, MO, USA). Cultures were then incubated for 3 days at 37℃, 5% CO_2. Cell proliferation was examined by MTS assay using the Celltiter 96® AQ_{ueous} One solution Cell Proliferation Assay Kit (Promega, Madison, WI, USA) following the manufacturer's instruction. The optical densities were measured in a microplate reader (Optimax, Molecular Devices, USA) at a wavelength of 490 nm. Absorbance was corrected by a prepared triplicate set of control wells (without cells) containing the same volumes of culture medium and CellTiter 96® AQ_{ueous} One Solution Reagent, as in the experimental wells. We subtracted the average 490 nm absorbance, from the "no cell" control wells, from all other absorbance values to yield corrected absorbances.

T cell subsets analysis

Spleen lymphocytes (1×10^6 cells/ml) were stained with fluoresein isothiocyanate (FITC)-labeled anti-mouse CD3 and phycoerythrin (PE)-labeled anti-mouse $CD4^+$ monoclonal antibodies for helper ($CD3^+$, $CD4^+$) T cells, or fluoresein isothiocyanate (FITC)-labeled anti-mouse CD3 and fluoresein isothiocyanate (FITC)-labeled

Table 2. Effects of dietary green tea by-products (GTB) on growth performances of finishing pigs.

Parameter	Control	Antibiotic	GTB-0.5%	GTB-1.0%	GTB-2.0%
Average initial weight (kg/pig)	77.20 ± 0.61	77.60 ± 0.69	77.47 ± 0.47	76.87 ± 0.71	77.07 ± 0.24
Average final weight (kg/pig)	113.33^{ab} ± 0.77	116.50^{a} ± 0.29	111.87^{ab} ± 2.07	112.80^{ab} ± 1.47	111.07^{b} ± 1.75
Average weight gain (kg/pig)	36.13^{ab} ± 1.37	38.90^{a} ± 0.98	34.40^{ab} ± 1.60	35.93^{ab} ± 1.29	34.00^{b} ± 1.51
Average feed intake (kg/pig)	131.67^{b} ± 8.82	47.50^{ab} ± 1.44	135.00^{b} ± 7.64	140.00^{ab} ± 7.69	158.20^{a} ± 0.92
Feed conversion ratio (feed/gain)	3.64^{b} ± 0.13	3.80^{b} ± 0.06	3.92^{b} ± 0.05	3.89^{b} ± 0.08	4.67^{a} ± 0.20

^{a,b}Means with different superscripts within same row are significantly different (P < 0.05). Data are presented as the mean ± SE.

anti-mouse CD8^{+} monoclonal antibodies for cytotoxic (CD3^{+}, CD8^{+}) T cells (Phar Mingen, San Diego, CA, USA) for 30 min at 4℃. The lymphocytes were collected by centrifugation at 160 ×g for 5 min and rinsed three times with PBS containing 10% FBS. The washed lymphocytes were fixed with 2% paraformaldehyde, and approximately 1 × 10^{4} cells were analyzed using a Coulter Epics XL Flow Cytometer (Beckman Coulter, Inc. CA, USA).

Cytokines (IL-6 and TNF-α) analysis

Splenocytes (5 × 10^{5} cells/ml) were cultured in 96-well flat-bottomed tissue culture plates in a final volume of 200 µl per well containing RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin, 1% (v/v) nonessential amino acids, and 0.05 mM 2-mercaptoethanol, and stimulated with LPS (10 µg/ml) or Con A (1.0 µg/ml) for 24 h. The cell culture supernatants were collected, stored at -20℃. The cell culture supernatants were assayed for IL-6 and TNF-α, using the Porcine IL-6 Quantikine ELISA kit (Cat. No. P6000) and Porcine TNF-α Quantikine ELISA Kit (Cat. No. PTA00) according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Optical density of each well was measured within 30 min, using a microplate reader (Optimax, Molecular Devices, USA) set to 450 nm (correction wavelength set at 570 nm). Each experiment was run in duplicate and the results represent means of three repeat experiments.

Statistical analysis

Data were analyzed using the general linear models of SAS (2003) to estimate variance components with a completely randomized design. Duncan's multiple comparison tests were used to examine significant differences among the treatment means. The level of significance was set at P < 0.05. Data are presented as mean values ± SE.

RESULTS

Growth performance, carcass characteristics and meat quality

Body weight, weight gain, feed intake and feed conversion ratio of different dietary groups are given in Table 2. The initial body weight did not differ significantly but the final body weight and body weight gain were lower (P < 0.05) in the GTB-2% group when compared to the antibiotic group. GTB-2% group exhibited a higher feed intake relative to GTB-0.5% and the control group and the FCR was also low when comparing to the other groups (P < 0.05). Different levels of GTB affect the proximate composition of loin meat (Table 3). When pigs were fed a diet containing GTB-2%, the crude fat decreased to the same level as the antibiotic group (P < 0.05). The GTB 0.5% group had a lower crude protein content, which was followed by the GTB-1% group (P < 0.05); however, higher value (GTB-2%) was not different than the antibiotic group.

Table 4 shows that the pigs in control group had a higher (P < 0.05) slaughter weight than the GTB-0.5 and 2% groups. There were no significant differences (P > 0.05) in back fat thickness, carcass grade, water holding capacity and pH among the groups; however, the shear value was higher in the GTB-0.5% group relative to the other GTB groups and the heating loss was lower in the GTB-2% group when compare to the others groups (P < 0.05). No differences in meat color, juiciness and flavor were observed due to supplementation with GTB, although the tenderness was lower in GTB-0.5% group (P < 0.05).

Biochemical and hematological parameters of blood and oxidative stability of meat

The effects of green tea by-products on the biochemical and hematological parameters of finishing pigs are presented in Table 5. There were no significant differences in total cholesterol, glucose, total protein, albumin, globulin, albumin/globulin (A/G) ratio, BUN and hemoglobin contents of the blood when the diets contained 0.5 to 2% of GTB. Although no significant differences were observed, the cholesterol level tended to decreased (P > 0.05) with an increase in the GTB content. WBC and RBC were increased (P < 0.05) at the highest GTB concentration (2%). TBARS values for the different treatment groups at different weeks are presented in Figure 1.

Significant differences (P < 0.05) were observed among treatments except fresh, 4th week and average

Table 3. Effects of dietary green tea by-products (GTB) on loin meat composition of finishing pigs.

Parameter (%)	Control	Antibiotic	GTB-0.5%	GTB-1.0%	GTB-2.0%
Moisture	72.95 ± 0.62	71.45 ± 0.62	72.27 ± 1.00	72.19 ± 0.42	73.53 ± 0.44
Crude ash	1.21 ± 0.03	1.13 ± 0.02	0.93 ± 0.05	1.14 ± 0.10	0.94 ± 0.16
Crude fat	2.10^{b} ± 0.35	2.66^{b} ± 0.28	4.21^{a} ± 0.19	3.87^{a} ± 0.20	2.19^{b} ± 0.34
Crude protein	23.04^{ab} ± 0.34	23.49^{a} ± 0.61	21.12^{c} ± 0.23	22.05^{bc} ± 0.38	23.27^{ab} ± 0.54

^{a,b,c}Means with different superscripts within same row are significantly different ($P < 0.05$). Data are presented as the mean ± SE.

Table 4. Effects of dietary green tea by-products (GTB) on carcass characters and meat quality in finishing pigs.

Parameter[1]	Control	Antibiotic	GTB-0.5%	GTB-1.0%	GTB-2.0%
Slaughter wt (kg/pig)	93.60^{a} ± 2.32	92.00^{ab} ± 1.73	85.17^{c} ± 2.85	91.33^{ab} ± 1.43	87.17^{b} ± 0.79
Back fat (mm)	19.60 ± 0.68	23.83 ± 1.08	21.83 ± 2.02	22.17 ± 1.82	21.17 ± 1.30
Carcass grade	1.80 ± 0.49	2.33 ± 0.33	2.67 ± 0.21	2.33 ± 0.42	2.67 ± 0.21
Shear value (kg)	3.14^{ab} ± 0.12	3.14^{ab} ± 0.10	3.38^{a} ± 0.19	2.97^{b} ± 0.05	2.86^{b} ± 0.07
Heating loss (%)	33.50^{a} ± 0.66	34.25^{a} ± 0.52	32.58^{a} ± 0.75	32.55^{a} ± 0.26	30.74^{b} ± 0.37
WHC (%)	57.34 ± 0.82	57.29 ± 0.16	56.83 ± 1.03	56.96 ± 0.55	57.91 ± 0.60
pH	5.78 ± 0.09	5.61 ± 0.01	5.66 ± 0.04	5.64 ± 0.05	5.64 ± 0.07
Meat color					
CIE *L*	51.54±1.10	54.24 ± 0.76	52.69 ± 3.18	54.55 ± 1.23	50.43 ± 1.81
CIE *a*	9.62 ± 0.54	9.98 ± 0.51	9.40 ± 1.19	9.32 ± 0.33	9.30 ± 0.35
CIE *b*	5.62 ± 0.68	6.47 ± 0.66	5.58 ± 0.94	6.50 ± 0.30	5.65 ± 0.90
Juiciness	3.73 ± 0.23	4.00 ± 0.12	3.80 ± 0.35	4.35 ± 0.22	4.08 ± 0.05
Tenderness	4.38^{a} ± 0.15	4.28^{ab} ± 0.17	3.70^{b} ± 0.34	4.35^{a} ± 0.05	4.48^{a} ± 0.13
Flavor	4.20 ± 0.12	4.05 ± 0.10	4.28 ± 0.18	4.25 ± 0.17	4.35 ± 0.05

^{a,b,c}Means with different superscripts within same row are significantly different ($P < 0.05$). Data are presented as the mean ± SE.
[1]The carcass grades were assessed on three points: 3, extremely good; 2, good; and 1, bad. WHC = water holding capacity; CIE = international commission on illumination; L = lightness; a = redness; b = yellowness.

values. These results demonstrated the consistency in the decrease of these values when higher levels of GTB were added. The antibiotic supplemented group showed a higher TBARS content in the 2nd week, whereas the TBARS content was higher in the control group during the 1st and 3rd week.

Spleen cells growth and cytokines production

Table 6 shows the immunity of spleen cells obtained from finishing pigs. When higher levels (1 to 2%) of GTB were added to the diet, the spleen weight was lower than the control group ($P < 0.05$). Production of helper and cytotoxic T cells among the dietary groups were not significant ($P > 0.05$), although higher values were seen in the GTB-0.5% and 1% group. As the dose of Con A and LPS increased, cytokine secretion from spleen cells increased. In both media, supplemented groups showed a higher value compare to the control group ($P < 0.05$). IL-6 and TNF-α production from spleen cells stimulated by LPS and Con A were positively influenced by dietary levels of GTB (Figure 2). When stimulated with 1.0 μg/ml Con A, IL-6 production was significantly increased in the GTB-1% group relative to the antibiotic and control group ($P < 0.05$), while IL-6 production by spleen cells with LPS (10.0 μg/ml) was higher in the GTB-2% group when compared to the GTB-0.5% and control group ($P < 0.05$). Data obtained from this experiment showed that TNF-α production from spleen cells treated with 1.0 μg/ml Con A and 10.0 μg/ml LPS were significantly higher for the GTB-1% and GTB-0.5% group, respectively, when compare to the antibiotic and control group ($P < 0.05$).

DISCUSSION

Body weights are commonly used for monitoring the nutritional status and growth of animals (Ndlovu et al., 2007). The different levels of green tea-by products in the experimental diets affected weight gain of the pigs. This result was supported by Yang et al. (2003) and Sayama

Table 5. Effects of dietary green tea by-products (GTB) on blood biochemical and hematological parameters in finishing pigs.

Parameter[1]	Control	Antibiotic	GTB-0.5%	GTB-1.0%	GTB-2.0%
Glucose (mg/dl)	52.10 ± 5.60	55.90 ± 3.63	60.70 ± 5.81	55.60 ± 7.52	65.20 ± 5.93
Cholesterol (mg/dl)	130.80 ± 5.37	123.00 ± 3.09	128.70 ± 5.45	123.30 ± 6.47	121.30 ± 5.96
Total protein (g/dl)	7.18 ± 0.20	7.49 ± 0.15	7.39 ± 0.22	7.34 ± 0.16	7.30 ± 0.25
Albumin (g/dl)	3.03 ± 0.17	3.25 ± 0.06	3.18 ± 0.13	3.10 ± 0.08	3.02 ± 0.13
Globulin (g/dl)	4.15 ± 0.11	4.24 ± 0.11	4.21 ± 0.12	4.24 ± 0.10	4.28 ± 0.16
A/G	0.74 ± 0.05	0.77 ± 0.02	0.76 ± 0.03	0.73 ± 0.02	0.71 ± 0.03
BUN (mg/dl)	17.70 ± 1.16	20.30 ± 0.82	18.30 ± 0.68	18.80 ± 1.11	18.50 ± 0.92
WBC (10^3/mm^3)	18.85^{bc} ± 0.46	18.57^{c} ± 0.55	20.45^{ab} ± 0.35	20.49^{ab} ± 0.73	20.87^{a} ± 0.67
RBC (10^6/ mm^3)	6.75^{b} ± 0.20	6.70^{b} ± 0.17	6.94^{ab} ± 0.047	7.21^{ab} ± 0.37	7.52^{a} ± 0.15
Hemoglobin (g/dl)	13.03 ± 0.19	13.08 ± 0.32	13.43 ± 0.15	13.68 ± 0.94	13.58 ±0.25

[a,b,c]Means with different superscripts within same row are significantly different ($P < 0.05$). Data are presented as the mean ± SE.[1]A/G = albumin/globulin; BUN = blood urea nitrogen; WBC = white blood cell; RBC = red blood cell.

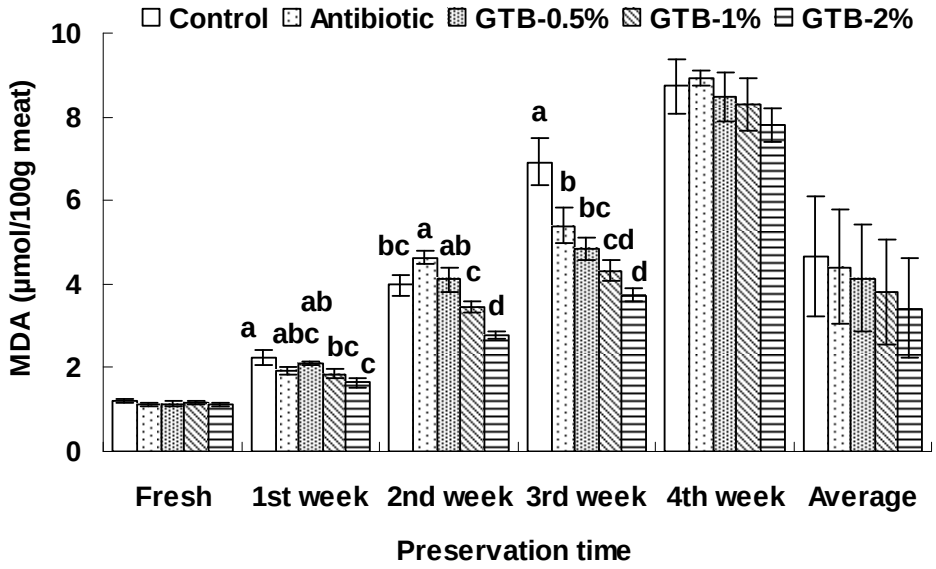

Figure 1. Effects of dietary green tea by-products (GTB) on thiobarbituric acid reactive substances (TBARS) values in loin meat. TBARS values were expressed as micromole of malondialdehyde (MDA) per 100 g of meat. Data are presented as the mean ± SE. Bars within a time class not sharing a common letter are significantly different ($P < 0.05$).

et al. (2000), who found that higher levels of green tea and body weight gain were negatively related in broilers and rats. In contrast, Ko et al. (2008) found no significant differences in the weight gain of finishing pigs when their diet contained GTB. In addition, Sarker et al. (2010) found a positive relationship in green tea probiotics when fed to weaning calves. In our experiment, feed intake and FCR were not affected up to 1% GTB. Ko and Yang (2008) and Shomali et al. (2011) found no significant differences in feed intake and FCR in pigs and broilers but Biswas and Wakita (2001) reported a lower feed intake and better FCR when 1% green tea powder was added to the feed for broilers. In general, the body weight gain and FCR tended to decrease with the addition of GTB. This was probably due to the high tannin content, which interferes with protein and starch digestion, and high fiber content of GTB. Some studies have reported that the catechin contents of green tea, mainly epicgallocatechin gallete, could inhibit digestive lipase activity and affect the lipid metabolism of animals (Sayama et al., 2000; Weisburger, 2001). Both crude protein and crude fat contents of meat were inversely proportional to each other, indicating that if the crude fat content was high then the crude protein content was low

Table 6. Effects of dietary green tea by-products (GTB) on spleen cell related immunity in finishing pigs.

Parameter	Control	Antibiotic	GTB-0.5%	GTB-1.0%	GTB-2.0%
Spleen weight (g)	181.50^{a} ± 24.02	145.75^{ab} ± 20.0	140.17^{ab} ± 20.01	132.42^{b} ± 5.42	132.50^{b} ± 9.38
Helper cell (%)	13.23 ± 0.87	12.88 ± 2.13	12.95 ± 0.74	13.12 ± 1.34	10.23 ± 1.38
Cytotoxic cell (%)	25.73 ± 2.18	23.63 ± 3.21	26.93 ± 1.56	26.33 ± 2.66	21.75 ± 2.05
Growth of spleen cells stimulated with concanavalin A (Con A)					
Con A-0.1 (µg/ml)	0.59^{b} ± 0.01	0.92^{a} ± 0.02	0.87^{a} ± 0.04	0.90^{a} ± 0.09	1.01^{a} ± 0.10
Con A -0.3 µg/ml)	0.66^{d} ± 0.01	0.94^{bc} ± 0.02	0.88^{c} ± 0.05	1.09^{a} ± 0.07	1.03^{ab} ± 0.05
Con A -1.0 µg/ml)	0.81^{b} ± 0.01	1.00^{a} ± 0.04	0.95^{ab} ± 0.05	1.10^{a} ± 0.09	1.00^{a} ± 0.04
Growth of spleen cells stimulated with lipopolysaccharide (LPS)					
LPS-1.0 µg/ml)	0.56^{b} ± 0.02	0.74^{a} ± 0.04	0.79^{a} ± 0.06	0.85^{a} ± 0.09	0.84^{a} ± 0.07
LPS-3.0 (µg/ml)	0.68^{b} ± 0.02	0.86^{ab} ± 0.02	0.85^{ab} ± 0.06	1.01^{a} ± 0.09	1.02^{a} ± 0.09
LPS-10.0 (µg/ml)	0.79^{b} ± 0.01	0.95^{b} ± 0.01	1.01^{ab} ± 0.08	1.02^{a} ± 0.09	1.26^{a} ± 0.15

^{a,b,c,d}Means with different superscripts within same row are significantly different (P < 0.05). Data are presented as the mean ± SE.

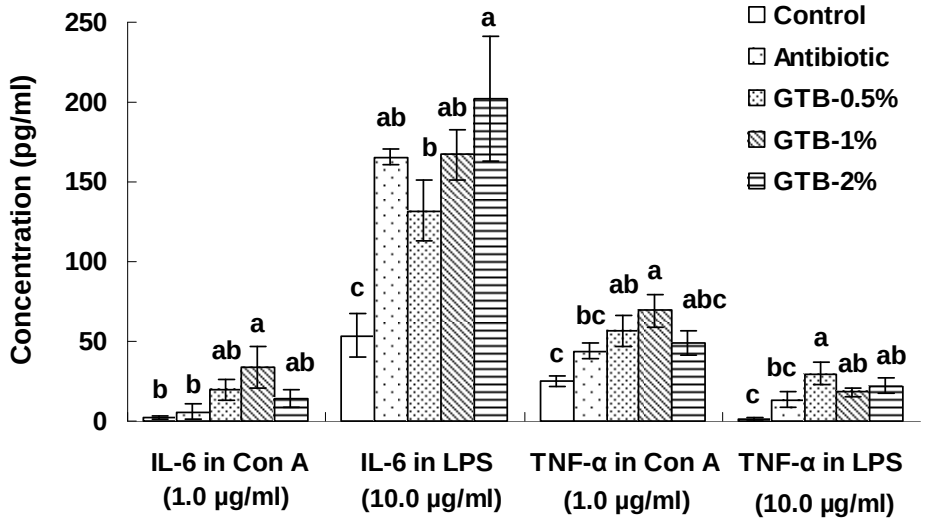

Figure 2. Effects of dietary green tea by-products (GTB) on IL-6 and TNF-α production by spleen cells in concanavalin A (Con A) and lipopolysaccharide (LPS) medium. Data are presented as the mean ± SE. Bars within a con A or LPS concentration not sharing a common letter are significantly different (P < 0.05).

(Davis et al., 1975). Ko and Yang (2008) observed the same phenomena when using green tea probiotics in the diet of finishing pigs. In this study, a similar result was observed for the GTB groups. Some researchers reported that the pigments included in the diets affect the color of meat and fat (Kim et al., 2006; Lee et al., 2009). It was believed that the yellowness of the meat and back fat increased when GTB was added to the diet since the pigments of GTB percolated through the meat. Lee (2005) reported that the addition of 0.02% green tea to the diet had no effects on back fat, carcass grade and meat color of the beef cattle. However, the addition of 2% green tea to the diet increased the yellowness and redness of the egg yolk (Uuganbayar et al., 2005). Jin et al. (2003) and Kim (2000) reported that the aroma, flavor, juiciness and overall acceptability of meat were affected by the ingredients of diets. Lee et al. (2009) also reported a positive effect of adding *Eucommia ulmoides* leaf to the diet in the sensory evaluation of meat. Although slaughter weight was some different, a positive effect on sensory evaluation and overall meat quality will be achieved by incorporation of GTB into the diet.

Biswas and Wakita (2001) and Kondo et al. (2004) observed a significant decrease in the serum cholesterol contents in broiler and lactating cow when broiler diets contained 0.5 to 1.5% green tea powder and 5% of GTB

in cow diet, which is similar to the results of this study. Green tea polyphenolics may favor the slow digestion of carbohydrates, which prevents sharp spikes of insulin in the blood and favors fat-burning over fat-storage (Zink, 2011). We observed no differences in the plasma biochemical composition with the addition of GTB, but some improvements in hematological parameters were observed. Lee (2005) found that supplementation of 0.02% GTB had no effect on the blood components in beef cattle. In contrast, Lee et al. (2009) found improve hematological and plasma biochemical parameters in pigs fed *E. ulmoides* leaves. However, Sarker et al. (2010) also found no significant differences among green tea probiotic and others group in regards to WBC, RBC albumin, globulin and A/G in pre and post weaning calves except albumin in the post-weaning period.

There is increasing interest in using the antioxidant compounds found in herbs and spices because they improve the flavor of food, retard the oxidative degradation of lipids and play an important role in the prevention of diseases (Achinewhu et al., 1995; Nakatani, 2000). Green tea studies showed that green tea extracts displayed a dose-dependent inhibitory activity against end stage of lipid peroxide decomposition product formation, and early lipid oxidation (Pearson et al., 1998; Yamane et al., 1999). Epigallocatechin gallate has been found to be over 100 times more effective in neutralizing free radicals than vitamin C and 25 times more powerful than vitamin E (Harold and Graham, 1992). It also has other antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene and resveratol. Ko et al. (2008) and Ko and Yang (2008) reported that meat obtained from pigs fed diets containing GTB and green tea probiotics had lower TBARS values, which was similar to the results observed in this study. Decreases in TBARS values were also found in broiler when the GTB content was increased from 0.5 to 1% level (Yang et al., 2003).

The spleen contains lymphocytes (mainly T cells and B cells) and macrophages, which engulf and destroy bacteria, dead tissue and foreign matter, and remove them from the blood passing through the spleen (Ezekowitz and Hoffman, 1998). Sayama et al. (2000) reported that 2% green tea supplementation to the rat diet depressed the spleen weight of the rats. The data obtained from current study showed that 0.5 to 2% GTB supplementation had an effect on spleen weight of finishing pigs. Pigs like other species contain $CD4^+$ and $CD8^+$ T lymphocytes in their peripheral blood and secondary lymphoid organs. These cells have been shown to express CD3 (Yang and Parkhouse, 1998) and have helper and cytolytic functions, respectively (Martins et al., 1993). However, unlike humans and mice, swine also have a prominent $CD4^+$ and $CD8^+$ lymphocyte population, comprising between 8 and 64% of the circulating pool of small resting T-lymphocytes (Zuckermann and Husmann, 1996). Helper and cytotoxic cells were numerically increased by the addition of 0.5 to 1% GTB in this study. The role of green tea components in improving cell-mediated and humoral immunity was previously investigated (Chae et al., 2004; Shin et al., 2004). Con A and LPS were shown to induce mitosis in T cells and B cells of many different specificities or colonial organ (Tanaka et al., 2005; Arens et al., 2004). Therefore, the growth reaction of spleen cells was checked by stimulating T cells with Con A and B cells with LPS, which specifically proliferates only T cells and B cells among the spleen cells. This experiment demonstrated that addition of GTB had a positive effect on humoral and cell-mediated immunity. Spleen cells secrete several types of cytokines in response to stimulation with Con A or LPS, which can be used to classify the immune response. One of the most important functions of IL-6 and TNF-α is the initiation of a response known as the acute-phase response. This involves a shift in the proteins secreted by the liver into the blood plasma and results from the action of IL-6 and TNF-α on hepatocytes (Kawai et al., 2004). The result of this experiment is an agreement with previous studies (Ko et al., 2008; Ko and Yang, 2008), who reported that the amount of IL-6 and TNF-α production by spleen cells increased when finishing pigs were fed diets containing GTB and green tea probiotics. It can be assumed that the bioactive components of green tea are responsible for these outcomes.

Conclusions

Based on the observations of this study, the addition of different GTB levels had an effect on some tested parameters. Growth performances were negatively and carcass composition was positively, affected at higher GTB concentrations (2%). Carcass characters, meat quality and hematological parameters did not consistently change with GTB concentration. Strong antioxidative activities were observed in the GTB groups. Our results also indicate that the addition of GTB to the diets of finishing pigs increases immune related cells, and enhances Il-6 and TNF-α production. Future studies are needed to elucidate the mechanisms for potentially enhanced immunity and to investigate its antimicrobial effect in challenged pigs.

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