



## Effect of Green Tea Probiotics on the Growth Performance, Meat Quality and Immune Response in Finishing Pigs

S. Y. Ko and C. J. Yang\*

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

**ABSTRACT :** The objective of this study was to determine the effects of green tea probiotics on growth performance, meat quality and immune response in finishing pigs, and to assess the possibility of substituting green tea probiotics for antibiotics in diets of finishing pigs. This green tea probiotics is made by mixing green tea powder and excipients (defatted rice bran and wheat bran) and fermenting the mixture with beneficial bacteria. A total of 90 crossbreed "Landrace×Yorkshire" finishing pigs with an average body weight of  $72.5 \pm 2.5$  kg were assigned to 5 dietary treatments in a completely randomized design. Each treatment had 3 replications with 6 pigs per replication. The five dietary treatments were control, antibiotic (0.003% chlortetracycline added) and 0.1, 0.5 and 1.0% of green tea probiotics. There were no significant differences in final body weight, daily weight gain, daily feed intake and feed conversion ratio in the green tea probiotics and antibiotic treatments ( $p > 0.05$ ). Crude protein content was significantly increased in the 0.1 and 1.0% green tea probiotics treatment groups ( $p < 0.05$ ) and there was no significant difference in crude fat content of the meat among the treatments. The TBA value of meat was significantly lowered with 0.5 and 1.0% green tea probiotics treatments compared to that of controls and statistically similar to the antibiotic treatment after 3 weeks of storage ( $p < 0.05$ ). The growth of spleen cells stimulated with Con A (0.1 and 1.0  $\mu\text{g/ml}$ ) was significantly increased with 1.0% green tea probiotics treatment compared to that of the control treatment ( $p < 0.05$ ). The growth of spleen cells stimulated with LPS (1.0, 3.0 and 10  $\mu\text{g/ml}$ ) was significantly increased in the 0.5% green tea probiotics group compared to the antibiotic group ( $p < 0.05$ ). In Con A (1.0  $\mu\text{g/ml}$ ) medium, IL-6 production of spleen cells was significantly increased with 1.0% green tea probiotics treatment compared to that of the control ( $p < 0.05$ ). In LPS (10.0  $\mu\text{g/ml}$ ) medium, TNF- $\alpha$  production of spleen cells increased significantly in all green tea probiotics treatment groups compared to that of the control ( $p < 0.05$ ). Finally it can be summarized that addition of green tea probiotic has a positive effect similar to antibiotic and 0.5% is the suitable dietary supplementation dose for finishing pig production. (**Key Words :** Green Tea, Probiotics, Growth Performance, Meat Quality, Immune Response, Pig)

## INTRODUCTION

Due to the occurrence of antibiotic-resistant bacteria and antibiotic residues in livestock products, the use of probiotics has been strongly recommended instead of antibiotics (Snyder and Champness, 1997). In comparison to antibiotics, probiotics are viable microorganisms that improve gut microflora by enzymes, organic acids, vitamins and nontoxic anti-bacterial substances that the microbes secrete once ingested by animals (Jun et al., 2002).

The development of probiotics for farm animals is based on the knowledge that the gut microflora is involved in resistance to disease. The stressful conditions experienced by young animals cause changes in the

composition and activity of gut microflora. Probiotic supplementation seeks to repair these deficiencies and provides the type of microflora that exists in feral animals uninfluenced by modern farm-rearing methods. *Lactobacillus*, together with *Bacillus sp.*, yeasts and filamentous fungi are the main components of probiotics commonly used for farm animals today (Fuller, 1989, 1999). It was observed that pigs receiving probiotics in their feed were equal to or superior in daily gain, intake, and feed efficiency when compared to pigs fed with antibiotics.

A recently developed non-antibiotic method is the use of functional medicinal plants (Berg, 1989; Harris and Webb, 1990; Martin and Nisbet, 1992; Lyons and Jacques, 2000; Kwon et al., 2005). Some examples of medicinal plants are green tea, artemisia, acanthopanax and others (Yang et al., 2003; Kwon et al., 2005). Green tea (*Camellia sinensis*) has been used for centuries by Korean, Japanese

\* Corresponding Author: C. J. Yang. Tel: +82-61-750-3235, Fax: +82-61-750-3239, E-mail: yangcj@sunchon.ac.kr  
Received October 13, 2007; Accepted May 13, 2008

**Table 1.** Formula and chemical composition of basal diet

Ingredients	%
Yellow corn	45.15
Wheat (13%)	25.00
Wheat bran	4.00
Soybean meal (40%)	16.00
Limestone	0.78
Calcium phosphate	1.10
Salt	0.25
Vitamin. premix <sup>1</sup>	0.55
Animal fat	2.50
Molasses	4.50
L-lysine	0.17
Chemical composition <sup>2</sup>	
ME (kcal/kg)	3,160
C. protein (%)	15.00
Ca (%)	0.78
Avail. P (%)	0.55
Lysine (%)	0.80
Methionine (%)	0.27

<sup>1</sup> Vitamin mix provided following nutrients per kg of premix: vitamin A, 6,000 IU; vitamin D<sub>3</sub>, 800 IU; vitamin E, 20 IU; vitamin K<sub>3</sub>, 2 mg; thiamin, 2 mg; riboflavin, 4 mg; vitamin B<sub>6</sub>, 2 mg; vitamin B<sub>12</sub>, 1 mg; pantothenic acid, 11 mg; niacin, 10 mg; biotin, 0.02 mg; Cu (copper sulfate), 21 mg; Fe (ferrous sulfate), 100 mg; Zn (zinc sulfate), 60 mg; Mn (manganese sulfate), 90 mg; I (calcium iodate), 1.0 mg; Co (cobalt nitrate), 0.3 mg; Se (sodium selenite), 0.3 mg.

<sup>2</sup> Calculated value.

and Chinese people as an anti-aging herb. It is reported that green tea and green tea catechins have many biological and biochemical effects such as anti-tumor effects (Itaro et al., 1988; Mukhtar and Ahmad, 1999), anti-oxidation (Mayumi et al., 1987), reduction in blood sugar and anti-angiogenesis (Matusuzaki and Hara, 1985; Ikeda et al., 1992). In addition to human consumption, low grade green tea has been used as an ingredient in animal feed for fish (Kono et al., 2000), broilers (Kaneko et al., 2001; Cao, 2005), calves (Ishihara et al., 2001) and pigs (Suzuki, 2002), hence the positive effects of green tea on animal performance have already been described. Bureenok et al. (2007) reported the positive effects on the increase of lactic acid bacteria and aerobic bacteria counts in ruminants. Kondo et al. (2006, 2007) reported that 10% FM of green tea waste added in silages for ruminant and goat diets with 5% of green tea by-product showed high nutritive values. Also, Nishida et al. (2006) reported that green tea waste silage to Holstein steers increased the concentrations of high density lipoprotein cholesterol.

This study was conducted with beneficial bacterial strains and a medicinal plant (green tea and green tea by-product) due to the limited research work which has been done in this field. Therefore, the objective of this study was to investigate the effects of green tea probiotics on growth performance, meat composition and immune response, and to assess the possibility of substituting green tea probiotics for antibiotics in the diet of finishing pigs.

**Table 2.** Microflora population and chemical composition of green tea probiotics

Items	Contents
Number of microflora of GI-P <sup>1</sup>	
<i>Lactobacillus acidophilus</i>	3.2×10 <sup>8</sup> cfu/g
<i>Lactobacillus plantarum</i>	2.2×10 <sup>8</sup> cfu/g
<i>Bacillus subtilis</i>	4.5×10 <sup>9</sup> cfu/g
<i>Saccharomyces cerevisiae</i>	5.2×10 <sup>8</sup> cfu/g
Chemical composition <sup>2</sup>	
Moisture (%)	15.08
C. Protein (%)	17.20
C. Fat (%)	4.93
C. Fiber (%)	10.89
C. Ash (%)	7.65
Total catechin (%)	4.76

The numbers represent the average value of the means. Each analysis was repeated three times (n = 3).

<sup>1</sup> Green tea probiotics, green tea comprised 30% of the total amount.

<sup>2</sup> Dry matter basis.

## MATERIAL AND METHODS

### Animal and experimental design

A total of 90 crossbreed (Landrace×Yorkshire) finishing pigs with an average initial body weight of 72.5±2.5 kg were housed in concrete floor pens. The pigs were assigned to 5 dietary treatments in a completely randomized design. Each treatment had 3 replicates with 6 pigs per replication. The five dietary treatments were control (no green tea probiotics added), antibiotics (control diet 0.003% chlortetracycline added), and diets containing 0.1, 0.5 and 1.0% green tea probiotic supplementations. The nutrient composition of the control diet (Table 1) was in accordance with recommendations for the nutrient requirements for finishing pigs (NRC, 1994). Green tea probiotics were produced through 2 steps: the first step was producing solid culture with 5 h of static and 3 h of shaking fermentation process by mixing 30% of green tea powder, 20% of wheat bran, and 50% of defatted rice bran inoculated by selected 2 strains Lactic acid bacteria (*Lactobacillus acidophilus* KCTC 3111 and *Lactobacillus plantarum* KCTC 3104). The second step was inoculating the selected 3 strains (*Bacillus subtilis* KCTC 3239, *Bacillus coagulans* KCTC 1015, and *Saccharomyces cerevisiae* KCTC 7915) with the solid culture and drying it. The chemical analysis of green tea probiotics showed crude protein, crude fat and crude fiber with the proportion of 17.20%, 4.93%, and 10.89% respectively, and the concentrations of microbes were 3.2×10<sup>8</sup> cfu/g of *Lactobacillus acidophilus*, 2.2×10<sup>8</sup> cfu/g of *Lactobacillus plantarum*, 4.5×10<sup>9</sup> cfu/g of *Bacillus subtilis* and 5.2×10<sup>8</sup> cfu/g of *Saccharomyces cerevisiae* (Table 2).

### Measurements and analysis

**Body weight :** The body weight of pigs was measured every two weeks from the initial day to the final day of the

experiment to calculate body weight gain.

**Feed intake and feed conversion ratio :** The feed intake of pigs was recorded every two weeks by offering a weighed quantity of feed and weighing their residues. The feed conversion ratio was calculated by dividing feed intake by the body weight gain of pigs.

**Meat composition, cholesterol and thiobarbituric acid (TBA) value :** Analyses of composition, cholesterol content and the thiobarbituric acid value (TBA) of loin meat were carried out at the end of the experiment. A total of 45 pigs were slaughtered taking 9 pigs from each treatment for analyses of meat composition, cholesterol and TBA value. TBA value was measured by each week of storage. The loin meat composition was analyzed by the methods of AOAC (1990). The cholesterol content of loin in porks was determined by the method of Brunnekreeft et al. (1983). Thiobarbituric acid value of meat was assayed by the methods of Vernon et al. (1970) with some modifications. For this analysis, 20 g loin mixture was blended with a cold extraction solution containing 20% trichloroacetate in 2 M phosphoric acid, and the slurry was allowed to precipitate. The supernatant was diluted to 100 ml DW and filtered through a Whatman No.1 paper. Then, 5 ml of the filtrate was transferred to a test tube (15×30 mm) where 5 ml of a 0.005 M 2-thiobarbituric solution was added. The solution was mixed by inversion and placed in a water bath at 80°C for 30 min. Once cooled, the resulting color was measured at 530 nm by a VIS-Spectrophotometer (Model 20D<sup>+</sup>, Milton Roy, USA).

**Immune response of spleen cells :** The analyses of immune response of spleen cells of pigs was done at the end of the experiment. The peripheral lymphatic organ of spleen is mainly composed of T cells, B cells and macrophages. Moreover, the antigen presenting cell acknowledges T cells and B cells of antigen invasion that leads to cellular and humoral immune responses. This is why spleen cells were used rather than lamina propria of the intestinal mucosa. At one third area of the spleens of pigs, a sample of tissue, size of 1 cm<sup>3</sup>, was extracted and it was split into a single cell on Bovine Serum Medium (RPMI-1640). After that, by using NycoPrep<sup>TM</sup> 1.077A, dead cells and red blood cells were removed and the number of surviving cells was counted

using Trypan blue. Spleen cells (5×10<sup>5</sup> cells/well) were transferred to a 96 well microplate containing LPS (1, 3 and 10 µg/ml) or Con A (0.1, 0.3 and 1.0 µg/ml), meeting the final volume of 200 µl in each well. Then the growth of the cells was measured after culturing in a 5% CO<sub>2</sub> incubator for 3 days. Respectively, cell growth was measured using a cell titer 96<sup>®</sup> aqueous one solution Cell Proliferation Assay (Promega Co., Madison, WI, USA), and 100 µl of the culture medium was removed and the remaining 100 µl was supplemented with 15 µl of cell titer. After culturing for 4 to 8 h, the optical density was measured at 490 nm using by a microplate reader (OPTImax, Molecular Devices, USA).

**Analysis of cytokine (IL-6 and TNF-α) on spleen cells :** For the antibodies of cytokine detection, IL-6 and TNF-α were used. Pig spleen cells were cultured for 24 h with LPS (10 µg/ml) and Con A (1.0 µg/ml) together, and the amount of IL-6 and TNF-α included in the upper fluid was measured by enzyme-linked immunosorbent assay (ELISA). The primary antibody and capture Ab were diluted in PBS, and 100 µl of it was transferred to plates respectively. After culturing for 24 h at 4°C, they were washed with a wash solution (0.05% Tween 20/PBS), and blocked for 2 h with a blocking solution (1% BSA, 5% sucrose, 0.05% NaN<sub>3</sub>). The previously cultured upper fluid was put together, and after 3 h, they were washed with the wash solution and a secondary detection antibody was added. After 2 h, the sample wells were washed and Streptavidin-HRP was supplemented. After one hour, the samples were washed, the substrate (2-azino-bis, 0.1 M citric acid, H<sub>2</sub>O<sub>2</sub>) added and, after chromogen, a microplate reader was used to measure the optical density at 450 nm. The measurement was converted using by a standard linear plot. The limit of measurement of each cytokine was 7.8 pg/ml.

### Statistical analysis

The data obtained from this study were analyzed by general linear models (GLM) of the SAS Package Program (1990) to estimate variance components for a completely randomized design. Duncan's multiple comparison tests (1955) were used to examine significant differences between treatment means. Differences were statistically assessed at  $p < 0.05$ .

**Table 3.** Effects of dietary green tea probiotics on the growth performance of pigs

Treatment	Control	Antibiotics	Green tea probiotics		
			0.1%	0.5%	1.0%
Initial body weight	71.06±0.86 <sup>a</sup>	70.33±0.88 <sup>ab</sup>	70.33±0.00 <sup>ab</sup>	69.72±0.51 <sup>b</sup>	71.00±0.17 <sup>a</sup>
Final body weight	110.44±0.84	112.61±2.04	112.33±0.33	111.67±1.69	111.78±2.08
ADG (kg)	0.93±0.00	1.01±0.04	1.00±0.01	0.99±0.03	0.97±0.04
ADFI (kg)	3.30±0.31	3.62±0.03	3.51±0.11	3.41±0.05	3.48±0.15
FCR (feed/gain)	3.54±0.33	3.58±0.14	3.51±0.13	3.44±0.05	3.59±0.29

Mean±standard error.

<sup>a,b</sup> Means with different superscripts within same row are significantly different ( $p < 0.05$ ).

**Table 4.** Effect of dietary green tea probiotics on meat composition of pigs (%)

Treatment	Control	Antibiotics	Green tea probiotics		
			0.1 %	0.5 %	1.0 %
Moisture	73.05±0.72	73.13±0.71	73.54±1.34	72.77±0.68	73.06±0.86
Crude protein	21.51±1.53 <sup>ab</sup>	20.81±1.15 <sup>ab</sup>	19.71±0.67 <sup>b</sup>	22.14±1.58 <sup>ab</sup>	22.58±1.58 <sup>a</sup>
Crude fat	1.83±0.65	2.87±0.66	2.70±0.39	2.53±0.57	1.75±0.78
Crude ash	1.87±0.14 <sup>a</sup>	1.41±0.14 <sup>b</sup>	1.53±0.21 <sup>b</sup>	1.37±0.21 <sup>b</sup>	1.27±0.09 <sup>b</sup>

Mean±standard error.

<sup>a, b</sup> Means with different superscripts within same row are significantly different ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### Growth performance

The effects of green tea probiotics on growth performance are shown in Table 3. There were no significant differences in final body weight, daily gain, daily feed intake and feed conversion ratio with 0.1, 0.5 and 1.0% green tea probiotics and antibiotic treatments. Supplementation of green tea probiotics tended to increase the average daily gain but no significant differences were observed.

Hines et al. (1971) and Cline et al. (1976) reported that microorganism additives have little effect on the feed for finishing pigs. Sayama et al. (2000) reported that weight gain of rats was reduced by adding 2.0 and 4.0% green tea extracts to their diet. The result of green tea probiotic supplementation in this experiment showed a similar effect to the experiment mentioned above. In general, in the animal industry, the benefits of herbs and other plant extracts can help improve feed intake, digestive enzymes and immunity reinforcement (Wenk, 2003).

### Composition, thiobarbituric acid value and cholesterol of meat

The effects of green tea probiotics on meat composition are shown in Table 4. The moisture content of the meat was not significantly different among treatment groups ( $p > 0.05$ ) and the crude protein content significantly increased in 0.1 and 1.0% green tea probiotics treatments ( $p < 0.05$ ). A tendency for increased crude protein content was seen as the green tea probiotics supplementation level was increased. The crude fat content of the meat was reduced with 1.0% green tea probiotics treatment compared to other groups, but there was no significant difference.

Davis et al. (1975) reported that the crude protein and the crude fat contents of meat are in inverse proportion to each other. In other words, if the crude fat content is high, the crude protein content is low. This result is similar to that of all groups treated with green tea probiotics in this experiment. Dulloo et al. (1999) reported that green tea and caffeine have thermogenin properties that promote fat oxidation and play a role in the control of body composition, probably through sympathetic activation of thermogenesis that could reduce obesity. These results are similar to those of our experiment which included 1.0% green tea probiotics. A similar study to the present experiment was done by Kim and Kim (2005) who demonstrated that when feeding pigs diets containing 1.0 to 5.0% persimmon peel powder, the highest crude fat content was found in the pigs fed diets containing a 5% inclusion level, and the crude protein content was not affected by the amount added, which is dissimilar to this trial.

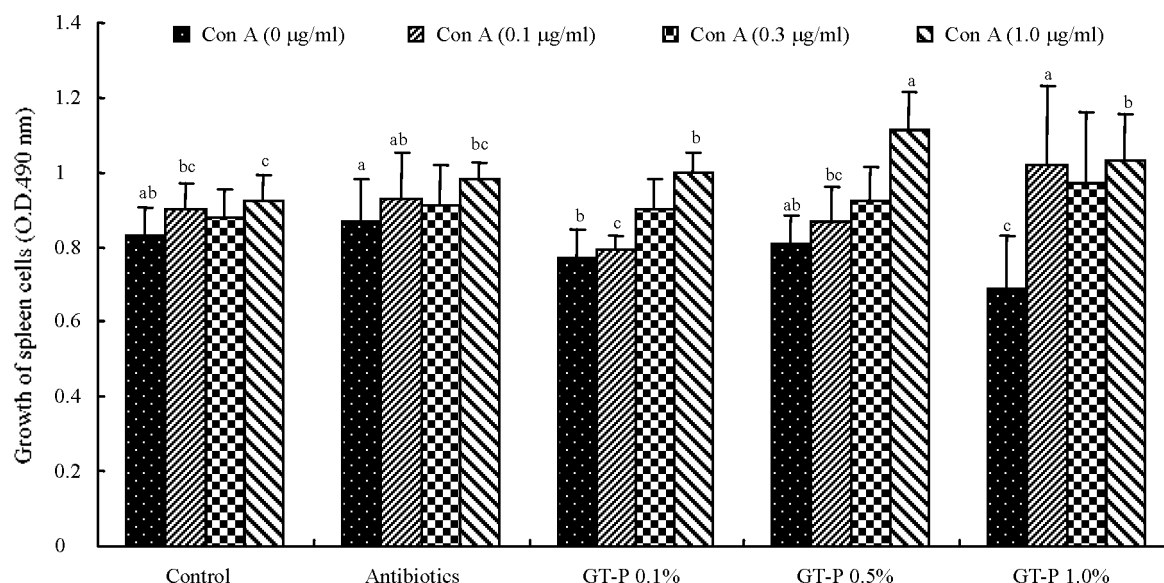
The effects of green tea probiotics on the meat TBA value are shown in Table 5. Diets containing 0.5 and 1.0% green tea probiotics supplements significantly lowered the TBA value of meat compared to the control ( $p < 0.05$ ). The incorporation of 1.0% green tea to the diet of rats reduced TBA values in the plasma (Yoshino et al., 1994). Catechin, a component of green tea, is considered to have dose-dependent inhibitory activity against the end stage of lipid peroxide decomposition product formation and early lipid oxidation (Pearson et al., 1998). A reduction in the TBA value of this experiment was obtained in the 0.5 and 1.0% green tea probiotic groups due to the high green tea content, a result that is similar to the experiment above. However, Kook and Kim (2003) reported that the TBA value increases when adding functional characteristic materials to the diet (Bamboo Vinegar) during the storage period, a

**Table 5.** Effect of dietary green tea probiotics on the TBA value of pigs ( $\mu\text{mol}/100\text{ g}$ )

Treatment	Control	Antibiotics	Green tea probiotics		
			0.1%	0.5%	1.0%
Fresh	1.02±0.09	1.32±0.38	1.42±0.28	1.32±0.23	1.25±0.09
1st week	1.82±0.28	1.97±0.32	2.06±0.40	1.85±0.90	1.85±0.24
2nd week	2.98±0.35	2.80±0.19	2.86±0.18	2.86±0.16	2.80±0.21
3rd week	4.46±0.23 <sup>a</sup>	3.97±0.16 <sup>bc</sup>	4.28±0.19 <sup>ab</sup>	3.94±0.11 <sup>c</sup>	3.97±0.19 <sup>bc</sup>

Mean±standard error.

<sup>a, b, c</sup> Means with different superscripts within same row are significantly different ( $p < 0.05$ ).



**Figure 1.** Effects of dietary green tea probiotics on growth of spleen cells stimulated with Con A (O.D. at 490 nm).

result dissimilar to ours.

The meat cholesterol content of green tea was 79.11, 80.14, 81.83, 78.02, and 81.20 mg/100 g for control, antibiotic and 0.1, 0.5, and 1.0% green tea probiotics group, respectively. There were no significant difference among the groups except for a slight decrease in the 0.5% green tea probiotics group.

Muramatsu et al. (1986) reported that 1.0 and 2.0% green tea extract with lard/cholesterol diets significantly reduced blood cholesterol level in rats. Sano et al. (1991) reported that 1.0% tea polyphenol in diets decreased significantly the serum cholesterol content in rats. It was demonstrated in other studies that high catechin contents in green tea may have an inhibitory effect on the intestinal absorption of lipid (Ikeda et al., 1992). Uganbayar et al. (2005, 2006) reported that 0.5 to 1.5% green tea supplementation in broiler diets and 1.0% to 2.0% green tea powder on layers had effects on reducing the cholesterol content of broiler meat and egg yolk of layers. These trial results are dissimilar to those obtained at inclusion levels from 0.1 to 1.0% in our experiment. That is, there was no significant difference based on the different levels of adding green tea probiotics.

#### Immune response of spleen cells in pigs

The spleen cells consist of T cells, B cells and macrophages. When antigens enter the body, the spleen notifies T and B cells of this intrusion. Thus, it stimulates cell-mediated and humoral immunity (Ezekowitz and Hoffman, 1998). Because T cells are important cells responsible for cell-mediated immunity, the role of green tea probiotics in improving cell-mediated immunity was previously investigated (Chae et al., 2004). Therefore, the

growth reaction of spleen cells was checked by stimulating T cells with Con A (concanavalin), which specifically proliferates only T cells among spleen cells.

The effects of green tea probiotics on growth of spleen cells stimulated by Con A (concanavalin) are shown in Figure 1. When stimulated by the largest dose of Con A (1.0 µg/ml), all treatments that contained green tea probiotics had a tendency to have a greater increase in immunity than control and antibiotic treatment, among which the 0.5% green tea probiotics group was the most effective. The growth of spleen cells stimulated with Con A (0.1 and 1.0 µg/ml) was significantly increased with the 1.0% green tea probiotics treatment compared to the control ( $p < 0.05$ ). In spite of Con A (1.0 µg/ml), the growth of spleen cells was significantly increased with 0.5% green tea probiotics compared to that of the antibiotics ( $p < 0.05$ ). However, the growth of spleen cells with Con A (0.3 µg/ml) medium was not significantly different among treatments ( $p > 0.05$ ).

B cells play an important role in humoral immunity which produces antibodies, and the effect of increased humoral immunity can be seen by the increase of B cells (Chae et al., 2004). Because B cells are important cells responsible for humoral immunity, the role of green tea probiotics in improving humoral immunity was previously examined (Shin et al., 2004). Therefore, spleen cell growth in reaction to green tea probiotics was checked by stimulating B cells with LPS (lipopolysaccharide), which specifically proliferates only B cells.

Effects of green tea probiotics on growth of spleen cells stimulated by LPS (lipopolysaccharide) are shown in Figure 2. The growth of spleen cells stimulated with LPS (1.0, 3.0 and 10 µg/ml) was significantly increased with 0.5% green

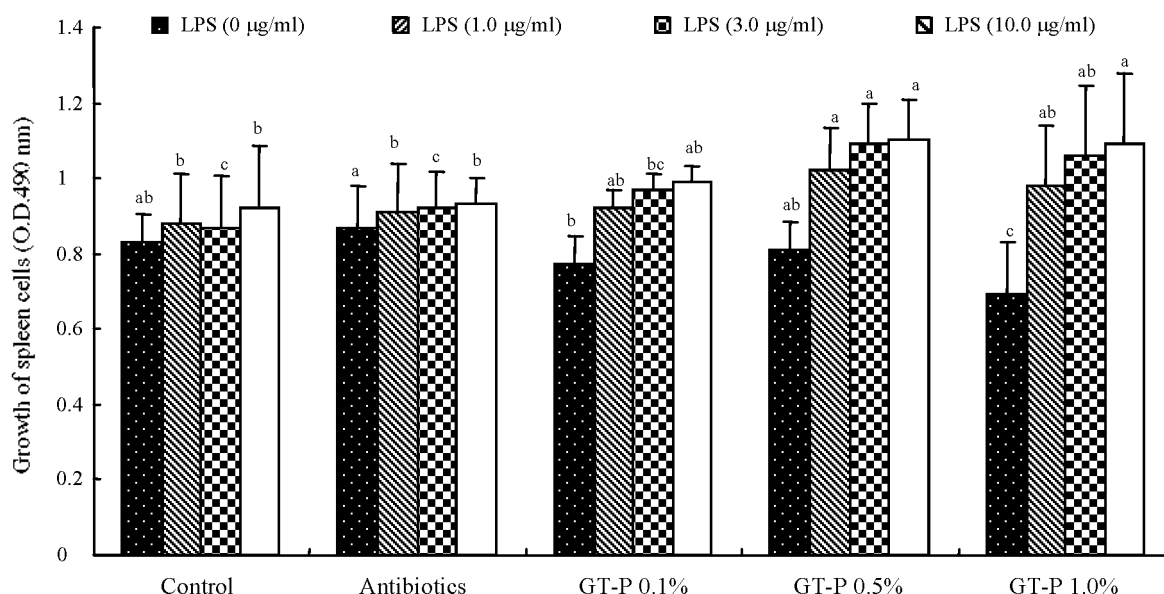


Figure 2. Effects of dietary green tea probiotics on growth of spleen cells stimulated with LPS (O.D. at 490 nm).

tea probiotics treatment compared to antibiotics ( $p < 0.05$ ). From these results, it may be recommended that adding 0.5% green tea probiotics into the diets of finishing pigs will have an effect on humoral immunity and cell-mediated immunity.

#### Production of cytokine (IL-6, TNF- $\alpha$ ) by spleen cells

Spleen cells secrete several types of cytokines when spleen cells are stimulated to grow in response to stimulation by Con A or LPS. If cytokines that are secreted during the growth reaction of spleen cells are analyzed, the type of immune response can be classified. Among these cytokines, IL-6 stimulates the synthesis of some blood

plasma proteins such as fibrinogen. Furthermore, IL-6 activates B cells which in turn activates antibody production (Devries, 1999).

The effects of dietary green tea probiotics on IL-6 production of spleen cells stimulated by LPS and Con A are shown in Figure 3. There were no significant differences in IL-6 production of spleen cells in LPS (10.0 µg/ml) medium among the different levels of green tea probiotics and antibiotic treatments ( $p > 0.05$ ). However, in Con A (1.0 µg/ml) medium, IL-6 production of spleen cells was significantly increased with 1.0% green tea probiotics treatment compared to the control ( $p < 0.05$ ), but it was not significantly different from that of the control ( $p > 0.05$ ).

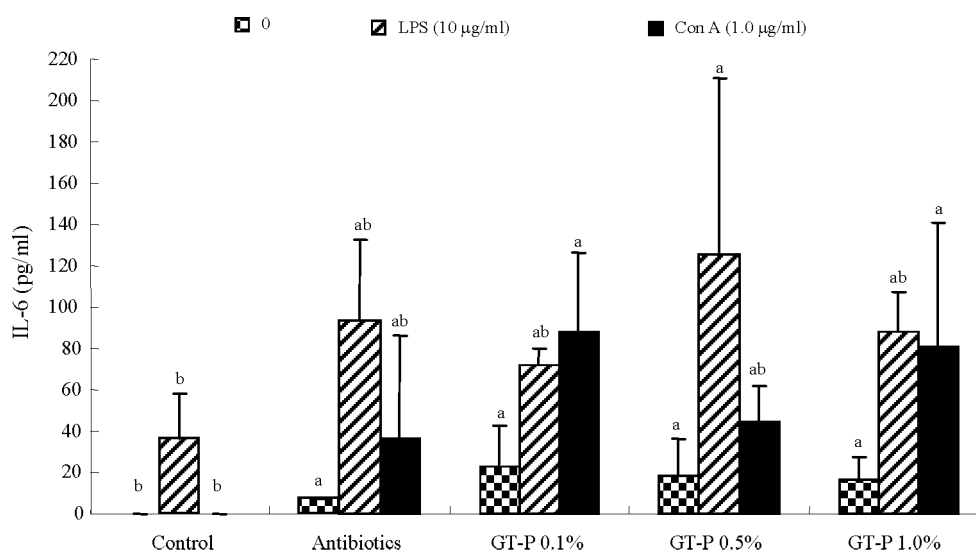


Figure 3. Effects of dietary green tea probiotics on IL-6 secretion by spleen cells (pg/ml).

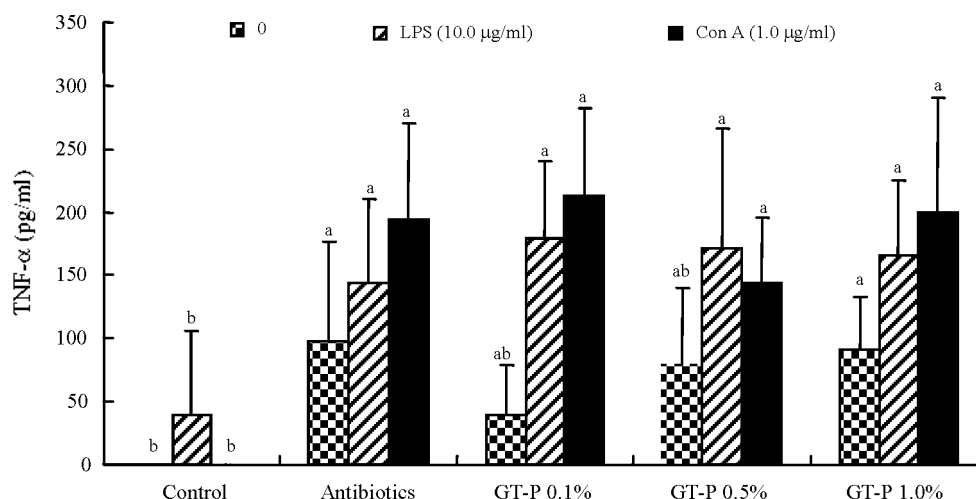


Figure 4. Effects of dietary green tea probiotics on TNF- $\alpha$  secretion by spleen cells (pg/ml).

When the growth reaction of spleen cells occurs, TNF- $\alpha$  is another cytokine that is secreted. TNF- $\alpha$  signals endothelial cells to express a new receptor site so that leukocytes obtain a capacity for adhesion and activates neutrophils to kill microorganisms (Schall and Bacon, 1994).

The effects of dietary green tea probiotics on TNF- $\alpha$  production of spleen cells stimulated by LPS and Con A are shown in Figure 4. In LPS (10.0  $\mu$ g/ml) medium, the TNF- $\alpha$  production of spleen cells increased significantly in 0.1, 0.5 and 1.0% green tea probiotics treatments compared to the control ( $p < 0.05$ ), but did not differ significantly from the antibiotic group ( $p > 0.05$ ). In the Con A group (1.0  $\mu$ g/ml), an identical tendency was observed as the TNF- $\alpha$  production of spleen cells was significantly higher with 0.1, 0.5 and 1.0% green tea probiotics treatments compared to the control but not different from the antibiotic group ( $p > 0.05$ ). Therefore, it is suggested that the amount of IL-6 and TNF- $\alpha$  secretion increases when the spleen cells of finishing pigs fed diets containing green tea probiotics are stimulated with Con A and LPS.

### IMPLICATIONS

The result of this study demonstrated that dietary addition of green tea probiotic has a positive effect on feed conversion efficiency, TBA value and immune responsiveness and can be used instead of antibiotic. A level of 0.5% green tea supplementation is the appropriate dietary dose for finishing pig production.

### ACNOWLEDGMENTS

This study was supported by the technology development program for Agriculture Forestry, Ministry of

Agriculture and Forestry, Republic of Korea. The author is highly appreciative for a subsidy from the Ministry of Agriculture and Forestry.

### REFERENCES

- AOAC. 1990. Official methods of analysis, 15th ed. Association of Official Analysis Chemist. Washington DC.
- Berg, R. D. 1998. Probiotics, prebiotics or conbiotics. Trends Microbiol 6:89-92.
- Brunnekreeft, J. W., G. J. Boerma and B. Leijnse. 1983. Direct determination of total cholesterol by on-column gas-chromatographic analysis without previous derivatisation compared with WHO-CDC reference method. Ann. Clin. Biochem. 20:360-363.
- Bureenok, S., M. Tamaki, Y. Kawamoto and T. Nakada. 2007. Additive effects of green tea on fermented juice of epiphytic lactic acid bacteria (FJLB) and the fermentative quality of rhodesgrass silage. Asian-Aust. J. Anim. Sci. 20:920-924.
- Cao, B. H., Y. Karasawa and Y. M. Guo. 2005. Effects of green tea polyphenols and fructooligosaccharides in semi-purified diets on broiler's performance and caecal microflora and their metabolites. Asian-Aust. J. Anim. Sci. 18:85-89.
- Chae, S. Y., S. H. Shin, M. J. Bae, M. H. Park, M. K. Song, S. J. Hwang and S. T. Yee. 2004. Effect of arabinoxylane and PSP on activation of immune cells. Kor. J. Soc. Food Sci. Nutr. 33(2):278-286.
- Cline, T. R., D. Forsyth and M. P. Plumlee. 1976. Probios for starter and grower pigs. Swine Day Rep. 53.
- Davis, G. W., G. C. Smith, Z. L. Capenter and H. R. Cross. 1975. Relationships of quality indicators to palatability attributes of pork loins. J. Anim. Sci. 41:1305.
- Devries, M. E., L. Ran and D. J. Kelvin. 1999. On the edge: the physiological and pathophysiological role of chemokines during inflammatory and immunological responses. Semin Immunol. 11:95-104.
- Dulloo, G., C. Duret, D. Ohrer, L. Girardier, N. Mensi, M. Fathi, P. Chantre and J. Vandermander. 1999. Efficacy of a green tea

- extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am. J. Clin. Nutr.* 70:1040-1045.
- Duncan, D. B. 1955. Multiple range and multiple F test. *Biometrics* 11:1.
- Ezekowitz, R. A. B. and J. Hoffman. 1998. Innate immunity. *Curr. Opin. Immunol.* 10:9-53.
- Fuller, R. 1989. Probiotics in man and animals. A Review. *J. Appl. Bacteriol.* 66:365-378.
- Fuller, R. 1999. Probiotics for farm animals. In: *Probiotics, A critical review, horizon scientific* (Ed. G. W. Tannock). England. 15-22.
- Harris, B. and D. W. Webb. 1990. The effect of feeding a concentrated yeast culture product to lactating dairy cows. *J. Dairy Sci.* 73 (Suppl. 1):266-266.
- Hines, R. H. and B. A. Koch. 1971. Response of growing and finishing swine to dietary source of *Lactobacillus acidophilus*. *Kansas Agr. Exp. Sta. Prog. Rep.* 181:29.
- Ikeda, I., Y. Ismasato and E. Sasaki. 1992. Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *J. Biochem. Biophys. Acta.* 1127:142-146.
- Ishihara, N., D. C. Chu, S. Akachi and L. R. Juneja. 2001. Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. *Livest. Prod. Sci.* 68:217-229.
- Itaro, O., N. Keiko and Y. Shigghiro. 1988. On the antitumor activity of fresh green tea leaf. *Agric. Biol. Chem.* 52:1879-1880.
- Jun, K. D., H. J. Kim, K. H. Lee, H. D. Paik and J. S. Kang. 2002. Characterization of *Bacillus polyfermenticus* SCD as a probiotic. *Kor. J. Microbiol. Biotechnol.* 30:359-366.
- Kaneko, K., K. Yamasaki, Y. Tagawa, M. Tokunaga, M. Tobisa and M. Furuse. 2001. Effects of dietary Japanese green tea powder on growth, meat ingredient and lipid accumulation in broilers. *Jpn. Poult. Sci.* 38:77-85.
- Kim, Y. J. and B. K. Kim. 2005. Effect of dietary persimmon peel powder on physico-chemical properties of pork. *Kor. J. Food Sci. Anim. Res.* 25(1):39-44.
- Kondo, M., K. Kita and H. Yokota. 2006. Evaluation of fermentation characteristics and nutritive value of green tea waste ensiled with byproducts mixture for ruminants. *Asian-Aust. J. Anim. Sci.* 19:533-540.
- Kondo, M., K. Kita and H. Yokota. 2007. Ensiled or oven-dried green tea by-product as protein feedstuffs: Effects of tannin on nutritive value in goats. *Asian-Aust. J. Anim. Sci.* 20:880-886.
- Kono, M., K. Furukawa, Y. M. Sagesaka, K. Nakagawa and K. Fujimoto. 2000. Effect of green tea grounds as dietary supplements on cultured yellowtail and ayu. *J. Jpn. Soc. Food Sci.* 47:932-937.
- Kook, K. and K. H. Kim. 2003. Changes in meat quality characteristics on refrigerated pork loin fed with supplemental bamboo vinegar. *Kor. J. Anim. Technol.* 45(2):265-272.
- Kwon, O. S., J. S. Yoo, B. J. Min, K. S. Son, J. H. Cho, H. J. Kim, Y. J. Chen and I. H. Kim. 2005. Effect of supplemental medicinal plants (*Artemisia*, *Acanthopanax* and *Garlic*) on growth performance and serum characteristics in lactating sows, suckling and weanling pigs. *Kor. J. Anim. Technol.* 47(4):501-512.
- Lyons, T. P. and K. A. Jacques. 2000. Biotechnology in the feed industry, Proceedings of Alltech's 16<sup>th</sup> Annual Symposium, Nottingham University Press.
- Martin, S. A. and D. J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. *J. Dairy Sci.* 75:1736.
- Matsuzaki, T. and Y. Hara. 1985. Antioxidative activity of tea leaf catechins. *Nippon Kaishi Nogeikagaku.* 59:129.
- Mayumi, F., M. Taeko and H. Yukhiko. 1987. Antioxidative activity of tea leaf catechins. *Proc. of the 1st International Symposium. Tea Quality Human Health.* Sept 17, Shizuoka, Japan. pp. 189-191.
- Mukhtatar, H. and N. Ahmad. 1999. Mechanism of cancer chemopreventive activity of green tea. *Proc. Soc. Exp. Biol. Med.* 220:234-238.
- Muramatsu, K., F. Maymi and H. Yokihiko. 1986. Effect of green tea catchiness on plasma cholesterol level in cholesterol fed rats. *J. Nutr. Sci. Vitaminol.* 32:613-622.
- Nishida, T., B. Eruden, K. Hosoda, K. Nakagawa, T. Miyazawa and S. Shioya. 2006. Effects of green tea (*Camellia sinensis*) waste silage and polyethylene glycol on ruminal fermentation and blood components in cattle. *Asian-Aust. J. Anim. Sci.* 19:1728-1736.
- NRC. 1994. Nutrient Requirements of Swine. 9th. Ed. National Academy Press. Washington, DC.
- Pearson, D. A., E. N. Frankel and R. Aeschbach. 1998. Inhibition of endothelial cell mediated low density lipoprotein oxidation by green tea extracts. *J. Agr. Food Chem.* 46:1445-1449.
- SAS. 1990. SAS<sup>®</sup> User's guide: statistics. Version 6, Fourth edition. SAS Institute Inc., Cary, NC.
- Sano, M., Y. Takashashi, C. Komatsu, Y. Nakamura, K. Shimoi, I. Tomita, I. Oguni, K. Nasu, K. Obata, H. Konomoto, T. Masuzawa and N. Suzuki. 1991. Antioxidative activities of green tea and black tea in rat organ, blood plasma. *Proc. of the International Symposium on Tea Science.* Shizuoka, Japan. 156.
- Sayama, K., S. Lin, G. Zheng and I. Oguni. 2000. Effect of green tea on growth, food utilization and lipid metabolism in mice. *In vivo.* 14:481-484.
- Schall, T. J. and K. B. Bacon. 1994. Chemokines, leukocyte trafficking, and inflammation. *Current Opinion in Immunology* 6:865-873.
- Shin, S. H., S. Y. Chae, M. H. Ha, S. K. Jo, S. H. Kim, M. W. Byun and S. T. Yee. 2004. Effect of Bu-Zhong-Yi-Qi-Tang on B cell development. *J. Kor. Soc. Food Sci. Nutr.* 33(2):271-277.
- Snyder and W. Champness. 1997. Molecular genetics of bacteria. *Am. Soc. Microbiol.* pp. 7-10.
- Suzuki, K., H. Kadowaki, M. Hino and K. Tamura. 2002. The influence of green tea in pig feed on meat production and quality. *Jpn. J. Swine Sci.* 39:59-65.
- Uganbayar, D., I. H. Bae, K. S. Choi, I. S. Shin, J. D. Firman and C. J. Yang. 2005. Effects of green tea powder on laying performance and egg quality in laying hens. *Asian-Aust. J. Anim. Sci.* 18:1769-1774.
- Uganbayar, D., I. S. Shin and C. J. Yang. 2006. Comparative performance of hens fed diets containing Korean, Japanese and Chinese green tea. *Asian-Aust. J. Anim. Sci.* 19:1190-1196.
- Vernon, C. W., G. F. Krause and E. M. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* 35:582-585.
- Wenk, C. 2003. Herbs and botanicals as feed additives in



- monogastric animals. *Asian-Aust. J. Anim. Sci.* 16:282-289.
- Yang, C. J., Y. C. Yang and D. Uuganbayar. 2003. Effect of feeding diets containing green tea by-products on laying performance and egg quality in hens. *Kor. J. Poult. Sci.* 30(3):183-189.
- Yoshino, K., M. Tomita, M. Sano, I. Oguni, Y. Hara and M. Nakano. 1994. Effects of long-term dietary supplement of tea polyphenols on lipid peroxide levels in rats. *Biol. Pharm. Bull.* 17:79-85.