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Comparison of the Effect of Green Tea By-product and Green Tea Probiotics on the Growth Performance, Meat Quality, and Immune Response of Finishing Pigs

S. Y. Ko, I. H. Bae, S. T. Yee[1], S. S. Lee, D. Uuganbayar, J. I. Oh and C. J. Yang*

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

ABSTRACT : The objective of this experiment was to compare the effects of green tea by-product and green tea probiotics on the growth performance, meat quality and immune response of finishing pigs. A total of 72 crossbred "Landrace×Yorkshire" finishing pigs with an average of 76 kg body weight were assigned to 4 dietary treatments in a completely randomized design. Each treatment had 3 replications with 6 pigs per replication. The four dietary treatments were control, antibiotics (control diet with 0.003% chlortetracycline added), and diets containing 0.5% green tea by-product or 0.5% green tea probiotic supplementation. Weight gain was increased in 0.5% green tea probiotics treatment compared to others, but there was no significant difference ($p>0.05$). The incorporation of 0.5% green tea probiotics to diets reduced the feed conversion ratio in finishing pigs ($p>0.05$). The incorporation of 0.5% green tea by-product into the pig diet reduced the crude protein and fat contents of the meat ($p>0.05$). Pigs fed diets containing 0.5% green tea probiotic supplementation had lowered meat TBA values compared to those fed 0.5% green tea by-product ($p<0.05$). The proliferation of spleen cells stimulated with Con A (concanavalin: 0.1, 0.3, and 1.0 μg/ml) significantly increased with 0.5% green tea by-product treatment compared to antibiotic treatment ($p<0.05$), but was significantly decreased in 0.5% green tea probiotics treatment compared to the antibiotic treatment ($p<0.05$). When stimulated with 1.0 μg/ml Con A, splenocyte production of IL-6 from pigs treated with 0.5% green tea by-product or green tea probiotics was significantly increased compared to the antibiotic treatment group ($p<0.05$). Splenocyte production of TNF-α after treatment with 1.0 μg/ml Con A was significantly higher following 0.5% green tea probiotics treatment ($p<0.05$), while TNF-α production after 10.0 μg/ml LPS (lipopolysaccharide) was significantly higher in the 0.5% antibiotic treatment group ($p<0.05$). (**Key Words** : Green Tea By-product, Green Tea Probiotics, Growth Performance, Meat Quality, Immune Response, Pig)

INTRODUCTION

There has been extensive use of antibiotics to prevent diseases and improve growth performance in the animal industry. However, due to the occurrence of antibiotic-resistant bacteria and antibiotic residue in livestock products, the use of probiotics has been strongly recommended instead of antibiotics (Snyder and Champness, 1997). Probiotics are viable microorganisms that improve gut microflora by enzymes, organic acids, vitamins and nontoxic anti-bacterial substances that the microbes secrete once ingested (Jun et al., 2002). Probiotic supplementation seeks to repair these deficiencies and provides the type of microflora that exists in feral animals uninfluenced by modern farm-rearing methods. Sedo (1986) compared the post-weaning performance of pigs fed starter feeds containing a microbial culture to that of pigs provided with a feed grade antibiotic. It was observed that the pigs receiving probiotics in their feed were equal to or superior in daily gain, intake and feed efficiency when compared to pigs fed antibiotics. There was an improvement of the growth performance not only of weaning pigs but also of growing finishing pigs by adding probiotics to feed (Baird, 1977). In addition, various kinds of fermented products using lactic acid bacteria are reported to have anticancer activities and enhance immunity (Kroger and Krumann, 1989; Itoh, 1999).

Another method that is developing together with probiotics is the recently developed non-antibiotic use of functional medicinal plants (Berg, 1998; Harris et al., 1990; Martin and Nisbet, 1992; Lyons and Jacques, 2000; Kwon

* Corresponding Author: C. J. Yang. Tel: +82-61-750-3235, Fax: +82-61-750-3239, E-mail: yangcj@sunchon.ac.kr

[1] Department of Life Science, Sunchon National University, Suncheon, 540-742, Korea.

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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[1]	0.55
Animal fat	2.50
Molasses	4.50
L-lysine	0.17
Chemical composition[2]	
ME (kcal/kg)	3,265
C. Protein (%)	16.00
Ca (%)	0.50
Avail. P (%)	0.45
Lysine (%)	0.80
Methionine (%)	0.27

[1] Vitamin.mix provided following nutrients per kg of premix: vitamin A, 6,000 IU; vitamin D_3, 800 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; thiamin, 2 mg; riboflavin, 4 mg; vitamin B_6, 2 mg; vitamin B_{12}, 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.
[2] Calculated value.

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 and Chinese people as an anti-aging herb. 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) and the positive effects of green tea on animal performance have already been described. The inclusion of green tea in broiler diets had positive effects on growth performance and lean meat production (Kaneko et al., 2001) and showed positive effects on the increase of lactic acid bacteria and aerobic bacteria counts in ruminants (Bureenok et al., 2007). However, utilizing green tea in the livestock industry has a negative effect in that it has a high cost. That is why the use of green tea by-product to substitute for green tea has been studied (Jung, 2001; Yang et al., 2003). 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. In addition, the green tea by-product is obtained through the production of green tea beverages and is recognized as cheap and effective for its utility, so it has been used as a feed supplement. Yang et al. (2003) reported that the TBA value of broiler meat decreased significantly when broilers were fed diets containing 0.5 to 2.0% green tea by-product supplementation compared to those fed a diet containing antibiotics. Nishida et al. (2006) reported that green tea waste silage to Holstein steers increased the concentrations of high density lipoprotein cholesterol. Currently, a method to develop a probiotic containing both beneficial bacterial strains and medicinal plants has not been studied.

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

Items	Contents
Number of microflora of GT-P[1]	
Lactobacillus acidophilus	3.2×10^8 cfu/g
Lactobacillus plantarum	2.2×10^8 cfu/g
Bacillus subtilis	4.5×10^9 cfu/g
Saccharomyces cerevisiae	5.2×10^8 cfu/g

The numbers represent the average value of the means. Each analysis was repeated three times (n = 3).
[1] Green tea probiotics, green tea comprised 30% of the total amount.

Therefore, the objective of this study was to compare the effects of green tea by-product and green tea probiotics on the growth performance, meat composition and immune response of finishing pigs.

MATERIALS AND METHODS

Animals and experimental design

A total of 72 crossbred (Landrace×Yorkshire) finishing pigs averaging 76 kg initial body weight were housed in concrete floor pens. The pigs were assigned to 4 dietary treatments in a completely randomized manner. Each treatment had 3 replicates with 6 pigs per replication due to experimental facility available in the farm. All animals were fed experimental diets for 4 weeks (28 days). The four dietary treatments were control (basal without antibiotics, green tea by-product and green tea probiotics added), antibiotics (0.003% chlortetracycline added), and diets containing 0.5% green tea by-product and 0.5% green tea probiotic supplementation. The nutrient composition of the control diet (Table 1) was in accordance with the suggestions by the Nutrient Requirements for swine (NRC, 1994). The green tea probiotics were produced through 2 steps; the first step was producing solid culture with 5 hours of static and 3 h of shaking fermentation process by mixing 30% green tea powder and 20% of wheat bran, and 50% of defatted rice bran inoculated by selected 2 strains (*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 concentrations of microbes were 3.2×10^8 cfu/g of *Lactobacillus acidophilus*, 2.2×10^8 cfu/g of *Lactobacillus plantarum*, 4.5×10^9 cfu/g of *Bacillus subtilis* and 5.2×10^8 cfu/g of *Saccharomyces cerevisiae* (Table 2).

Table 3. Effects of dietary green tea by-product and green tea probiotics on the growth performance of pigs

Parameters \ Treatments	Control	Antibiotics	GTB[1] 0.5%	GTP[2] 0.5%
Initial body weight (kg)	75.19±1.09	76.52±0.86	77.06±1.76	76.80±1.79
Final body weight (kg)	101.43±1.88	102.75±3.68	102.09±3.03	104.25±2.66
Weight gain (kg)	26.24±1.96	26.23±3.28	25.04±3.51	27.45±0.98
Feed intake (kg)	91.29±7.74	88.19±3.46	84.49±7.13	89.02±7.80
FCR (feed/gain)	3.50±0.44	3.41±0.48	3.45±0.67	3.25±0.36

Data: means±standard error. [1] Green tea by-product. [2] Green tea probiotics.

Measurements and analysis

Body weight, feed intake and feed conversion ratios were measured every two weeks. Analyses of cholesterol content, the thiobarbituric acid value (TBA) of the loin meat, and immune responses by pig spleen cell were carried out after the end of growth performance experiment.

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 body weight gain of pigs.

Meat composition, cholesterol and thiobarbituric acid (TBA) values : Analyses of meat composition, cholesterol content, and the thiobarbituric acid value (TBA) of loin porks were carried out at the end of the experiment. A total of 45 pigs were slaughtered taking 9 pigs from each treatment for the analyses of meat composition, cholesterol and TBA value. TBA value was measured by each week of storage. The composition of loin in porks was analyzed by the common methods of AOAC (1990). The meat cholesterol 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 Whatman No.1 paper. Then 5 ml of the filtrate was transferred to a test tube (15×30 mm) where 5 ml of 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$^+$, Milton Roy, USA).

Immune response of spleen cells : The analyses of immune response of spleen cells of pigs were done at the end of the experiment. The peripheral lymphatic organ of spleen is mainly composed of T cells, B cells and macrophages. Moreover, antigen presenting cell acknowledges T and B cells of antigen invasion that leads to cellular and humoral immune response. 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 tissue, size of 1 cm^3, was extracted and it was split into a single cell on Bovine Serum Medium (RPMI-1640). After that, by using NycoPrep™ 1.077A, dead cells and red blood cells were removed and the number of surviving cells was counted using Trypan blue. Spleen cells (5×10^5 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_2 incubator for 3 days. Respectively, cell growth was measured using a cell titer 96® 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) production by spleen cells : IL-6 and TNF-α were used for the antibodies of cytokine detection. The spleen cells of pigs 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, was diluted in PBS and 100 μl of it was transferred to plates respectively. After incubating for 24 h at 4°C, the wells were washed with a wash solution (PBS/0.05% Tween 20) and blocked for 2 hours with a blocking solution (1% BSA, 5% sucrose, 0.05% NaN_3). The previously cultured upper fluid was put together and after 3 h, they were washed with the wash solution and a secondary detection Ab was added. After 2 h, the sample wells were washed and Streptavidin-HRP was supplemented. After an hour, the samples were washed, substrates (2-azino-bis, 0.1 M citric acid, H_2O_2) added, and after chromogen, a microplate reader was used to measure the optical density at 450 nm. The measurement was converted using a standard plot. The limit of measurement in individual cytokine was 7.8 pg/ml.

Table 4. Effect of dietary green tea by-product and green tea probiotics on meat composition (%)

Parameters \ Treatments	Control	Antibiotics	GTB[1] 0.5%	GTP[2] 0.5%
Moisture	72.35±0.43	72.49±0.38	71.55±0.67	71.56±0.79
Crude protein	24.04±1.34	24.25±0.67	23.34±0.71	24.62±0.38
Crude fat	1.58±0.24	1.47±0.39	1.36±0.54	1.43±0.65
Crude ash	1.30±0.16	1.22±0.02	1.24±0.05	1.29±0.05

Data: means±standard error. [1] Green tea by-product. [2] Green tea probiotics.

Statistical analysis

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

RESULTS AND DISCUSSION

Growth performance

The effects of green tea by-product and green tea probiotics on growth performance are shown in Table 3. There were no significant differences in final body weight, weight gain, feed intake or feed conversion ratio in groups containing 0.5% of green tea by-product and green tea probiotic treatments, and antibiotic treatment ($p>0.05$). Weight gain was increased with 0.5% green tea probiotics treatment compared to others but there was no significant difference ($p>0.05$). The incorporation of 0.5% green tea probiotics in pig diets reduced the feed conversion ratio in finishing pigs ($p>0.05$).

Sayama et al. (2000) reported that the weight gain of rats was reduced with 2.0 and 4.0% green tea addition to rat feed. Yang et al. (2003) reported that 1.0 and 2.0% green tea by-product addition to a broiler diet reduced the weight gain of chicks. In our experiment, a similar result was obtained with 0.5% green tea by-product. Mavromaitis and Kyriakis (1998) suggested that by adding oreganum essential oil, feed intake and feed conversion ratio were improved. The inclusion of 0.5% green tea probiotics in this experiment showed similar results to the above experiment.

Compositions and thiobarbituric acid values of meat

The effects of green tea by-product and green tea probiotics on meat composition are shown in Table 4. The moisture content of the meat exhibited no significant differences among treatment groups ($p>0.05$). The incorporation of 0.5% green tea by-product in the pig diet reduced the crude protein and crude fat contents of the meat in finishing pigs ($p>0.05$), with no significant differences among the dietary treatment groups.

Davis et al. (1975) reported that both crude protein and crude fat contents of meat were inversely proportional to each other, indicating that if the crude fat content is high then the crude protein content is low. In our experiment, the 0.5% green tea probiotics treated group demonstrated a similar result. Park et al. (2003) reported on an experiment of feeding finishing pigs with 0.1 and 0.4% yeast culture containing *Saccharomyces cerevisiae*. Among the two groups, the 0.4% treatment group had higher crude protein and lower crude fat contents. This result is similar to our result with the inclusion of 0.5% green tea probiotics.

The effects of green tea by-product and green tea probiotics on meat TBA values are shown in Table 5. The TBA value of meat was not affected by dietary supplementation with 0.5% green tea by-product and green tea probiotics to finishing pigs ($p>0.05$). Pigs fed diets containing 0.5% green tea probiotic supplementation had a lower meat TBA value compared to that of 0.5% green tea by-product treatment ($p<0.05$).

Yang et al. (2003) reported that broilers fed diets containing 0.5 to 1.5% green tea by-product had lower TBA values of meat than those treated with antibiotic supplement. This result does not seem to be similar to the result we observed with 0.5% green tea by-product in this experiment. However, Kook and Kim (2003) reported that the TBA value increased when adding functional characteristic materials (Bamboo Vinegar) to pork during the storage

Table 5. Effect of dietary green tea by-product and green tea probiotics on the TBA value of meat (μmol/100 g)

Storage period \ Treatments	Control	Antibiotics	GTB[1] 0.5%	GTP[2] 0.5%
Fresh	0.74±0.46	0.80±0.19	0.52±0.11	0.28±0.16
1st week	1.66±0.28[a]	1.08±0.19[b]	1.02±0.18[b]	1.08±0.19[b]
2nd week	8.44±4.01	7.32±1.34	7.80±2.29	7.52±3.67
3rd week	16.60±8.82	14.55±0.92	16.26±4.62	15.88±2.93

Data: means±standard error.

[a, b] Means with different superscripts within same row are significantly different ($p<0.05$).

[1] Green tea by-product. [2] Green tea probiotics.

Table 6. Effect of dietary green tea by-product and green tea probiotics on the cholesterol content of meat (mg/100 g)

Parameter \ Treatments	Control	Antibiotics	GTB[1] 0.5%	GTP[2] 0.5%
Cholesterol	65.70±2.48	63.10±4.18	60.78±6.66	60.16±6.16

Data: means±standard error. [1] Green tea by-product. [2] Green tea probiotics.

period. This result is similar to the result of treatment with 0.5% green tea by-product in this experiment.

Cholesterol of meat

The effects of green tea by-product and green tea probiotics on meat cholesterol content are shown in Table 6. The total cholesterol content of meat was slightly decreased in the 0.5% green tea by-product and green tea probiotics treatment groups compared to the antibiotic group, but no significant difference among treatments was found ($p>0.05$).

Uuganbayar 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. Jung (2001) reported that the cholesterol content of broiler meat was decreased when broilers were fed 0.5 to 2.0% green tea by-product supplemented diets. These results are similar to the result of 0.5% green tea by-product and green tea probiotics treatments in this experiment.

Kim et al. (2000) observed a significant decrease in the cholesterol content of meat when feeding 0.1 to 0.5% probiotics-containing feed, with *Bacillus sp.* and *Lactobacillus sp.*, to broiler chicks. This result is similar to that of the 0.5% green tea probiotics treatment in this experiment.

Immune responses of spleen cells in pigs

Spleen cells consist of T cells, B cells and macrophages. When antigens intrude into the body, the spleen notifies T and B cells, thus stimulating cell-mediated immunity and humoral immunity (Ezekowitz and Hoffman, 1998). T cells are responsible for cell-mediated immunity, and a role for green tea by-product and green tea probiotics in improving cell-mediated immunity has been observed (Chae et al., 2004). Therefore, the growth response of spleen cells was measured by stimulating T cells with Con A (concanavalin), which specifically causes the proliferation of T cells.

The effects of green tea by-product and green tea probiotics on the growth of spleen cells stimulated by Con A are shown in Figure 1. The growth of spleen cells stimulated with Con A (0.1, 0.3 and 1.0 μg/ml) significantly increased with 0.5% green tea by-product treatment compared to antibiotic treatment ($p<0.05$), but it was significantly decreased with 0.5% green tea probiotics treatment compared to antibiotic treatment ($p<0.05$). Our study demonstrated that 0.5% green tea by-product inclusion in the finishing diet had positive effects on the cell-mediated immunity of pigs.

B cells play a crucial role in humoral immunity which produces antibodies, and the effect of increase in humoral immunity can be observed by the increase of B cells (Chae et al., 2004). Green tea by-product and green tea probiotics

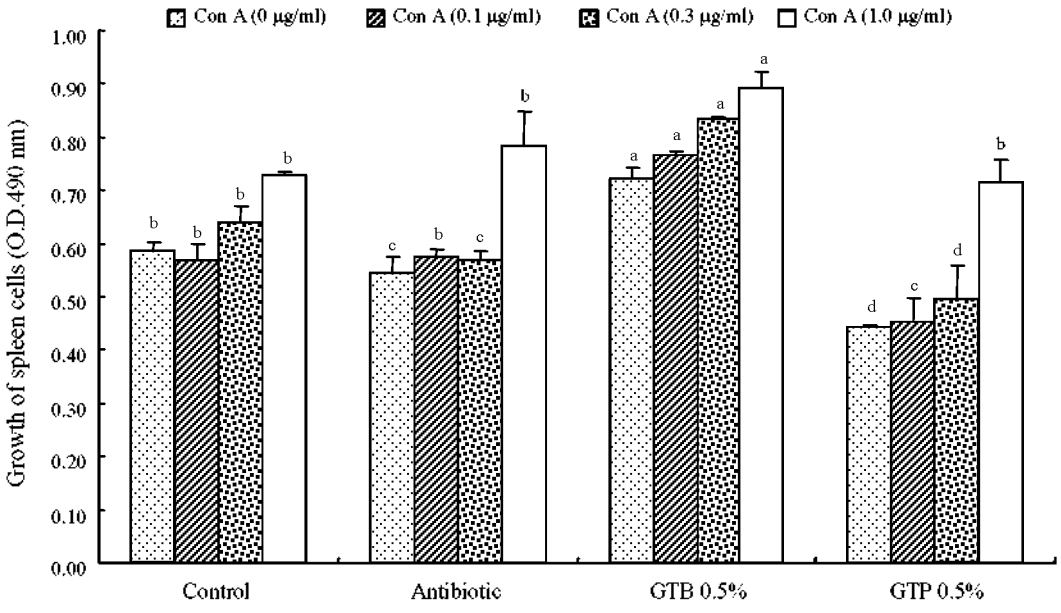

Figure 1. Effects of dietary green tea by-product and green tea probiotics on growth of spleen cells stimulated with Con A.

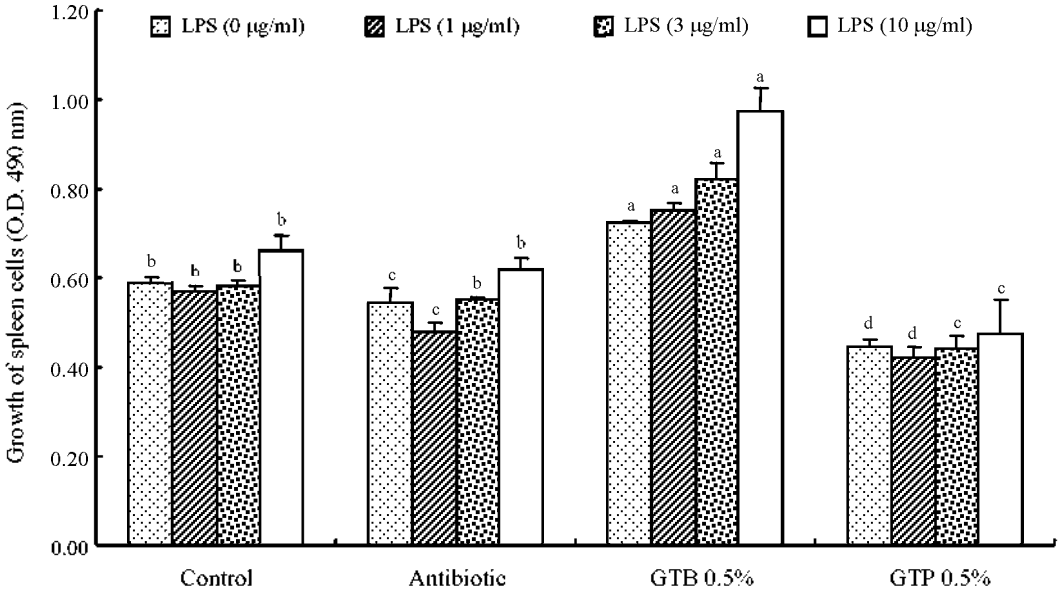

Figure 2. Effects of dietary green tea by-product and green tea probiotics on growth of spleen cells stimulated with LPS.

were shown to improve humoral immunity (Shin et al., 2004). Therefore, the effect of green tea by-product and green tea probiotics was checked by stimulating B cells with LPS (lipopolysaccharide), which specifically causes the proliferation of B cells.

The effects of green tea by-product and green tea probiotics on the growth of spleen cells stimulated by LPS are shown in Figure 2. When spleen cells were stimulated with 1.0, 3.0, or 10.0 μg/ml LPS, spleen proliferation was significantly increased in the 0.5% green tea by-product treatment group ($p<0.05$). In contrast, it was significantly decreased in the 0.5% green tea probiotics group compared to the antibiotic treatment group ($p<0.05$). Therefore, our study demonstrated that the inclusion of 0.5% green tea by-product in the finishing diet had positive effects on the humoral immunity of pigs.

Production of cytokines (IL-6, TNF-α) by spleen cells

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. IL-6 stimulates the synthesis of some blood plasma proteins such as fibrinogen. It also activates B cells and functions as a B cell growth factor, which leads to antibody production (Devries et al., 1999). The effects of dietary green tea by-product and green tea probiotics on IL-6 production of spleen cells stimulated by LPS and Con A are shown in Figure 3. When stimulated with LPS (10.0 μg/ml), IL-6 production of spleen cells was

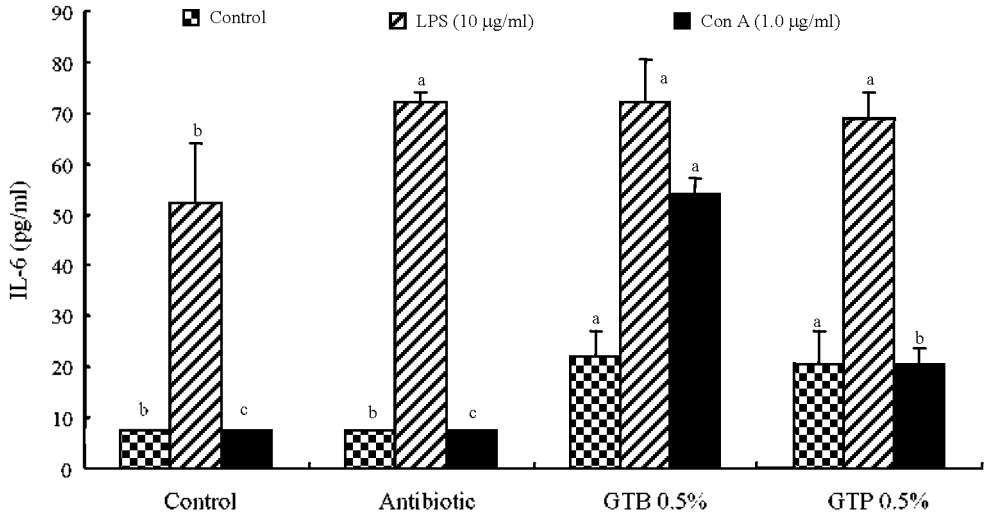

Figure 3. Effects of dietary green tea by-product and green tea probiotics on IL-6 secretion by spleen cells.

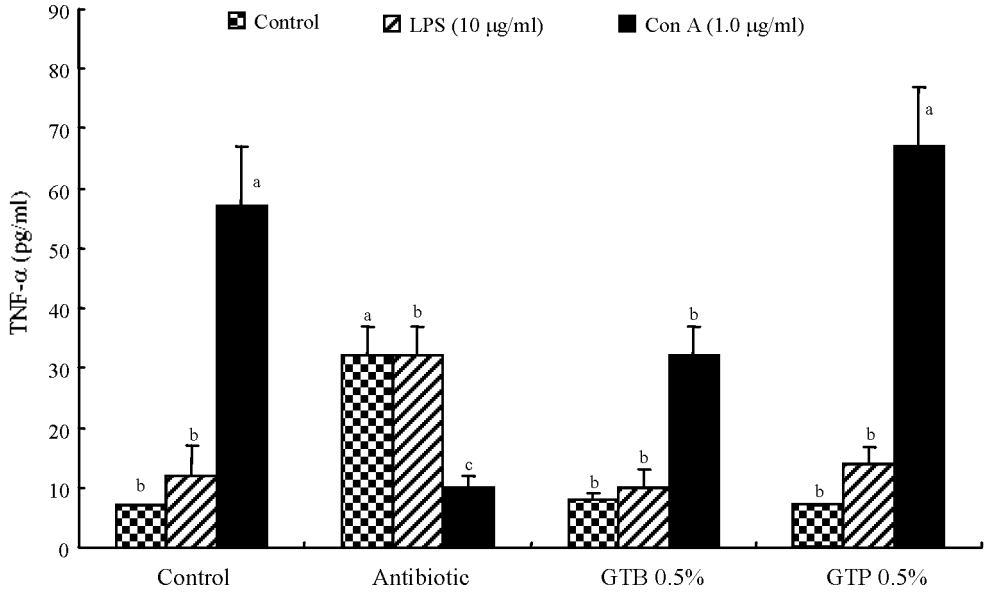

Figure 4. Effects of dietary green tea by-product and green tea probiotics on TNF-α secretion by spleen cells.

significantly increased in green tea by-product and green tea probiotics treatment groups compared to the control ($p<0.05$). When stimulated with 1.0 μg/ml Con A, IL-6 production was significantly increased in 0.5% green tea by-product or green tea probiotics treatment groups compared to the antibiotic treatment group ($p<0.05$).

When spleen cells are activated, another cytokine that spleen cells secrete is TNF-α. It induces endothelial cells to express receptors so that leukocytes can adhere, and activates neutrophils to kill microorganisms (Schall and Bacon, 1994). The effects of dietary green tea by-product and green tea probiotics on TNF-α production of spleen cells stimulated by LPS and Con A are shown in Figure 4. TNF-α spleen cell production with 1.0 μg/ml Con A was significantly higher with 0.5% green tea probiotics treatment ($p<0.05$), while TNF-α production with 10.0 μg/ml LPS was significantly higher with 0.5% green tea by-product treatment compared to antibiotic treatment ($p<0.05$). In LPS (10 μg/ml) medium, TNF-α production of spleen cells was significantly increased with 0.5% green tea probiotics treatment compared to the antibiotic treatment; however, in Con A (1.0 μg/ml) medium, TNF-α production of spleen cells did not differ from other treatments ($p<0.05$). Therefore, we observed that the amount of IL-6 and TNF-α secretion increases when spleens of finishing pigs fed diets containing 0.5% green tea by-product or green tea probiotics are stimulated with Con A and LPS.

IMPLICATIONS

The results of our study demonstrate that the inclusion of 0.5% green tea by-product and green tea probiotics in finishing pig diet improves growth performance and meat quality. Also, our results support that adding 0.5% green tea by-product and green tea probiotics to the diets of finishing pigs will have an effect on humoral and cell-mediated immunity.

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