Journal of Medicinal Plants Research Vol. 4(7), pp. 546-550, 4 April, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.501
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

Effects of *Camellia sinensis* and mixed probiotics on the growth performance and body composition in broiler

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Accepted 4 January, 2010

This study was conducted to investigate the effects of green tea and fermented green tea probiotics on the growth performance and body composition in broiler chicks. A total 168 Ross broilers chicks, one-day old was randomly allotted to 6 treatments with 4 replications having 7 layers per replication. There were six dietary treatments namely negative control (without antibiotics), positive control (basal + 30 ppm oxytetracycline), green tea (basal + 0.5% and basal + 1.0%) and fermented green tea probiotics, FGTP (basal + 0.5% and basal + 1.0%). The results revealed that body weight gain and feed conversion ratio of broiler chicks were not significant among the treatments. The crude protein content of broiler meat was increased significantly in 1.0% FGTP group compared to the other treatments (P < 0.05). The thiobarbituric acid (TBA) value of broiler meat in 0.5% green tea showed lowest than other groups though no significant differences (P > 0.05) were observed except fresh and 1st week after preservation. In conclusion, diets containing 0.5% green tea and 1.0% FGTP groups were suitable for broiler growth performance and meat composition.

Key words: Green tea, fermented green tea probiotics, broiler, weight gain, crude protein, lipid oxidation.

INTRODUCTION

There has been extensive use of antibiotics to prevent diseases and improve growth performance in the animal industry. However, due to the outbreaks of resistant bacteria and residues of antibiotics in poultry products, using antibiotics is regulated by Korean Government (Yu et al., 2004). There are several kinds of antibiotics alternatively developed and used currently, among which readily memorable natural substances for customers and medicinal plants with excellent physiologically activity are getting attention by researchers (Chen et al., 2003; Hernandez et al., 2004). Some examples of medicinal plants are green tea, artemisia, acanthopanax and others (Yang et al., 2003; Kwon et al., 2005; Sarker et al., 2010). Green tea (*Camellia sinensis*) containing the most effective antioxidant, catechin has been used for centuries by Korean, Japanese and Chinese people as an anti-aging herb. Green tea inclusion in broiler diets had positive effects on growth performance and lean meat production of the broilers (Kaneko et al., 2001). In addition to human consumption, low grade green tea has been used as an ingredient in broilers (Kaneko et al., 2001; Cao, 2005).

Probiotics, with medicinal plants, is being suggested as effective antibiotics alternatives. Fuller (1989) defined probiotics as a microbial feed supplement which gives beneficial effects to the host by improving the balance of microbes in intestines.

Probiotics maintains intestinal microbial balance and helps gut mucosa development, improving digestion and absorption rate and thus improving production (Mohan et al., 1996). It has been reported by several studies that single and mixed probiotics increases poultry production (Jin et al., 1996; Mohan et al., 1996). Generally, the digestive organ of healthy animal is dominated by anaerobic organisms and the upper part of it is dominated by *Lactobacillus acidophilus*, the middle part by *Bacillus*

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subtilis and the lower part by S. faecium. Considering this fact, the effect of probiotics may be improved using mixed probiotics (Yoon et al., 2004). It is reported that supplementation of 0.1 and 0.2% of mixed probiotics containing *L. acidophilus*, *B. subtilis* and *Saccharomyces cervisae* improves production of broilers and indirect immunity (Kim et al., 2002).

The ultimate goal of antibiotics alternatives is a complete substitution of antibiotics. A complete substitution by one substance, however, is difficult in reality. With these reasons, studies on synergistic effects using two alternative substances with different mechanism are newly emerging. Therefore, the objective of this study is to evaluate the effects on production and body composition of broilers when fed fermented green tea probiotics using only green tea and green tea with beneficial microbes.

MATERIALS AND METHODS

Animals and experimental design

One hundred sixty eight "Ross" broiler chicks of one-day old were used for this study. The chicks were housed in a close, ventilated caged-broiler house in which they were raised for 24 h of daily light. From 1 to 14 days of age, supplemental heat was provided by electric heater which placed inside the chicken house, thereafter the room temperature was kept at 22 ± 2°C through a supplemental heating system.

The birds were assigned to 6 treatments with 4 replications having 7 broiler chicks per replication following completely randomized design (CRD). There were six dietary treatments, control, antibiotic (basal + 30 ppm oxytetracycline), green tea (basal + 0.5% and basal + 1.0%) and fermented green tea probiotics (basal + 0.5% and basal + 1.0%). The feed and drinking water were provided ad libittum.

Experimental diets and feeding

Experimental diets were divided into two phases; starter 0 - 3 weeks and finisher 4 - 5 weeks of age. Diets were formulated following NRC (1998). The chemical compositions of experimental diets are given in Table 1.

The chemical composition of green tea and fermented green tea probiotics are given in Table 2. This fermented green tea supplement is a probiotics that was made by mixing green tea powder and excipients (defatted rice bran and wheat bran) and fermenting the mixture with beneficial bacteria. Fermented green tea supplement were produced as follows: Lactic acid bacteria (*L. acidophilus* KCTC 3111 and Lactobacillus plantarum KCTC 3104) were formulated into media containing 10% green tea, 60% defatted rice bran and 30% wheat bran.

It was, then, fermented at 40°C for 5 h in anaerobic conditions and 3 h in aerobic conditions and it was subsequently continued for 48 h. After that, there was a second inoculation with bacteria (B. subtilis KCTC 3239) and yeast (S. cerevisiae KCTC 7915), then dried. This is composed of 19.20% of crude protein, 2.92% crude fat, 11.08% of crude fiber in the fermented green tea supplement. Also, 4.2×10^7 cfu/g of L. acidophilus, 5.8×10^6 cfu/g of L. plantarum, 2.6×10^7 cfu/g of B. subtilis and 6.2×10^9 cfu/g of S. cerevisiae were included in fermented green tea supplement.

Table 1. Chemical composition of broiler experimental diets (%).

Chemical composition	Broiler starter	Broiler finisher
ME (kcal/kg)	3,100	3,150
Crude protein (%)	22.00	19.00
Crude fat (%)	4.00	4.50
Crude ash (%)	8.00	8.00
Crude fiber (%)	6.00	6.00
Methionine (%)	0.79	0.70
Ca (%)	0.80	0.75
P (%)	0.54	0.52

Table 2. The number of microflora population and chemical composition of green tea and fermented green tea probiotics.

Item	Content (cfu/g)
Number of microflora of FGTP[1)]	
Lactobacillus acidophilus	4.2×10^7
Lactobacillus plantarum	5.8×10^6
Bacillus subtilis	2.6×10^7
Saccharomyces cerevisiae	6.2×10^9

Item	Green tea	FGTP
Chemical composition (%)		
Moisture	11.60	12.40
Crude protein	22.36	19.20
Crude fat	7.36	2.92
Crude fiber	16.20	11.08
Crude ash	6.22	10.63

The numbers are represented the average value of the means [1)] FGTP- Fermented green tea probiotics, green tea was taken 10% of the total amount.

Parameters studied

Body weight gain, feed intake, feed conversion ratio, weight of internal organs, body composition, lipid oxidation of broiler meat (equal amount of breast and thigh meat), caecal microbe measurement.

Statistical analysis

The data obtained from this study were analyzed by general linear models (GLM) of 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 $P < 0.05$.

RESULTS AND DISCUSSION

Body weight

The results revealed that body weight gain, feed conversion

Table 3. Effects of green tea and mixed probiotics on growth performance and feed intake of broilers chicks.

Items	Control	Antibiotics	Green tea		FGTP	
			0.5%	1.0%	0.5%	1.0%
Initial weight (g)	44.82	44.86	44.71	44.79	45.14	44.93
Final weight (g)	2079.29	2073.57	2087.86	1883.57	2092.86	2054.29
Weight gain (g)	2034.46	2028.71	2043.14	1838.79	2047.71	2009.36
Feed intake (g)	3453.99	3423.43	3462.29	3172.46	3435.46	3472.96
FCR (feed/gain)	1.70	1.69	1.70	1.73	1.68	1.73

[a,b,c] Mean within the same rows are not significantly different (P > 0.05).

Table 4. The effects of green tea and fermented green tea probiotics on body composition of broiler chicks (%).

Items	Control	Antibiotics	Green tea		FGTP	
			0.5%	1.0%	0.5%	1.0%
Moisture	74.92^{a}	73.74^{ab}	73.69^{ab}	73.07^{bc}	73.53^{ab}	71.85^{c}
Crude protein	23.61^{b}	22.11^{b}	23.55^{b}	22.06^{b}	23.56^{b}	26.41^{a}
Crude fat	0.71	0.85	1.25	1.53	1.04	1.37
Crude ash	1.10^{b}	1.10^{b}	1.13^{ab}	1.13^{ab}	1.17^{a}	1.17^{a}

[a, b, c] Mean with different superscripts within the same rows are significantly different (P < 0.05).

ratio and feed intake of broiler chicks were not significant among the treatment groups (Table 3).

In the case of 1.0% green tea and 1.0% fermented green tea probiotics, the body weight gain was reduced compared to the others. These results were similar with Kaneko et al. (2001) who reported that 1.0, 2.5 and 5.0% of green tea in broiler diets linearly reduced body weight gain of the chicks and Uuganbayar (2004) reported that 1.0 to 1.5% green tea supplement in broiler diet had effect to reduce body weight gain of the chicks. Kim et al. (2002) reported that it was effective to improve weight gain and feed conversion ratio when 0.1 to 0.5% probiotics with *Lactobacillus sp., Bacillus sp.* and Yeast was fed to broilers. According to Ryu et al. (2003), supplementation of 0.1 to 0.3% mixed probiotics containing *L. acidophilus*, *B. subtilis*, *S. cervisae* to broiler chicks fed diets improved weight gain and feed conversion ratio as well as feed intake. Uuganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet had effect to reduce body weight gain and feed intake of the chicks. It is considered that the difference of growth performance between 0.5 and 1.0% fermented green tea supplement treatment groups of our study is originated from the composition of green tea.

Body composition

The meat composition of broilers is given in Table 4. The moisture content of broiler meat was significantly lower in 1.0% fermented green tea supplement group compared to the other treatment groups (P < 0.05) and the crude protein was increased significantly in 1.0% fermented green tea supplement group compared to the other treatment (P < 0.05).

The crude fat content was reduced in control and antibiotics groups compared to others, but there was no significant difference (P > 0.05). The crude ash increased significantly in broilers fed diets containing 0.5 and 1.0% of fermented green tea supplement groups compared to the control and antibiotics groups (P < 0.05). Davis et al. (1975) reported that the crude protein content and the crude fat of meat are negatively correlated to each other. In other words, if the crude fat content is higher, the crude protein content tended to lower.

Lipid oxidation of meat

The thiobarbituric acid (TBA) test is the most widely used method for quantifying lipid oxidation development in meat and meat products. The TBA test determines the amount of malondialdehyde (MDA), a major secondary by-product of lipid oxidation, in an oxidized lipid. Lipid oxidation has been reported to be a primary cause of off-odor and significant correlations between the TBA values and sensory scores of poultry meat have been reported. In Table 5, TBA value of fresh broiler meat was significantly lower in 0.5% green tea supplement group (1.69 umol/100 g)**,** while control was significantly high (2.31 umol/100 g) (P < 0.05). In case of one week of storage, TBA value of broiler meat was significantly higher compared to the other treatment groups (P < 0.05). The average TBA value of 0 to 3 weeks of storage period,

Table 5. The effects of green tea and fermented green tea probiotics on the TBA value of broiler meat (umol MDA/100 g).

Storage period	Control	Antibiotics	Green tea		FGTP	
			0.5%	1.0%	0.5%	1.0%
Fresh	2.31^{a}	2.06^{abc}	1.69^{c}	2.22^{ab}	1.82^{bc}	1.94^{abc}
1st week	4.52^{a}	2.77^{b}	2.55^{b}	3.54^{ab}	3.20^{b}	3.11^{b}
2nd week	7.28	6.55	6.43	6.49	7.26	7.11
3rd week	15.02	14.62	13.88	13.57	14.40	14.03
Average	7.28	6.50	6.14	6.45	6.67	6.55

$^{a, b,c}$ Mean with different superscripts within the same rows are significantly different (P < 0.05).

Table 6. The effects of feeding green tea and fermented green tea probiotics on the development of internal organs of broiler chicks.

Items	Control	Antibiotics	Green tea		FGTP	
			0.5%	1.0%	0.5%	1.0%
Crop wt.	0.29	0.30	0.26	0.27	0.23	0.22
Heart wt.	0.60	0.60	0.65	0.64	0.58	0.62
Liver wt.	1.87^{ab}	1.86^{ab}	1.91^{ab}	2.20^{a}	1.69^{b}	1.74^{b}
Gizzard wt.	0.89	1.01	0.94	0.96	0.93	0.88
Pancreas wt.	0.16^{ab}	0.17^{ab}	0.16^{ab}	0.14^{b}	0.18^{ab}	0.20^{a}
Cecum wt.	0.49^{ab}	0.55^{ab}	0.37^{b}	0.61^{a}	0.39^{b}	0.44^{ab}
Kidney wt.	0.75	0.70	0.74	0.79	0.72	0.67
Small intestine wt.	2.59^{a}	2.33^{ab}	1.98^{b}	2.60^{a}	1.99^{b}	2.20^{ab}
Large intestine wt.	0.16	0.18	0.16	0.16	0.16	0.18
Abdominal fat wt.	1.84^{ab}	1.91^{ab}	2.12^{ab}	1.39^{b}	2.99^{a}	2.26^{ab}
Proventriculus wt.	0.38	0.40	0.40	0.33	0.32	0.31

$^{a, b,c}$ Mean with different superscripts within the same rows are significantly different (P < 0.05). LW, Live weight; FGTP, Fermented green tea probiotics.

the TBA value of broiler meat in 0.5% green tea showed the lowest value among the groups, although no significant difference (P > 0.05) was observed.

Uuganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet had effects to reduce the TBA value on broiler meat compared to the control and Yang et al. (2003) also reported that the TBA value of broiler meat was decreased significantly when broilers were fed diets with 0.5 to 2.0% green tea by-products supplement diet. Mountney (1976) reported that the rancidity of broiler meat arises faster than the red meat of porks and beef because of more unsaturated fatty acid contained. It can be concluded that the green tea supplementation in broiler diet may reduce the rancidity of the broiler meat.

Weight of internal organs

The results showed (Table 6) that the liver weight was increased significantly in broilers fed diets containing 1.0% green tea supplement (P < 0.05). The pancreas weight was increased significantly in broilers fed diets containing 1.0% fermented green tea supplement compared to the other treatment groups (P < 0.05).

The results showed that the cecum weight was significantly reduced in broilers fed diets containing 0.5% green tea supplement compared to the other treatment groups (P < 0.05). The abdominal fat weight was reduced significantly for broilers fed diets containing 1.0% of green tea supplementation (P < 0.05). The results showed that the small intestine weight was reduced significantly for the broilers fed diets containing 0.5% green tea and 0.5% fermented green tea supplement supplementation (P < 0.05). Uuganbayar (2004) reported diets containing 0.5% green tea showed a significant weight loss of the small intestine compared to the control diet, which is similar to our study. Kim et al. (2006) also expressed similar opinion.

Cecal microbe measurement

The results showed (Table 7) that the total bacterial count was tended to increase in all treatments group more than the control group. The total cecal bacterial count was the highest in 1.0% fermented green tea probiotics group of

Table 7. The effects of green tea and green tea probiotics on the change of cecal microbe of broiler chicks.

	Control	Antibiotics	Green tea		FGTP	
			0.5%	1.0%	0.5%	1.0%
Total bacterial	3.4×10^7	4.1×10^7	3.6×10^7	3.8×10^7	2.7×10^8	3.2×10^8
Lactobacillus spp.	3.6×10^6	2.8×10^6	3.0×10^7	4.3×10^6	4.8×10^7	4.6×10^8
E. coli	3.1×10^5	2.7×10^5	4.6×10^4	3.2×10^4	2.5×10^4	3.8×10^4

The numbers are represented the average value of the means. Each analysis was replicated by three times (n=3).

3.2×10^8, the lowest in the control group of 3.4×10^7. The number of *Lactobacillus spp*. in 1.0% FGTP group was the highest, recording 4.6×10^8, while the antibiotics group of 2.8×10^6 was the lowest. The number of E. coli was tended to reduce in green tea and fermented green tea supplement treatment groups compared to the control and antibiotics groups. Antimicrobial activity mechanism is still not clear, but the excellence of medicinal plants and plant extracts is widely known. Guo et al. (2004) and Lucy (2002) reported that herb polysaccharides extract increases the number of beneficial bacteria, *Lactobacilli* and decreases harmful bacteria, E. coli, to balance the number of intestinal microbes. Their reports are similar to the result of our study.

Considering the parameters, diets containing 0.5% green tea and 1.0% fermented green tea probiotics supplementation were found suitable for broiler growth performance and meat composition.

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