Effects of Green Tea Polyphenols and Fructo-oligosaccharides in Semi-purified Diets on Broilers' Performance and Caecal Microflora and Their Metabolites

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ABSTRACT : This study was conducted to examine the effects of green tea polyphenols (GTP) and fructo-oligosaccharides (FOS) supplement on performance, counts of caecal microflora and its metabolites production. In female broiler chickens fed on semi-purified diets from 28 to 42 d of age, dietary green tea polyphenols (GTP) and fructo-oligosaccharides (FOS) significantly reduced mortality ($p<0.05$). Dietary GTP significantly decreased the total count of caecal microflora, each colonic population count and caecal flora metabolites contents when compared to other groups ($p<0.05$). Dietary FOS did not influence the total count of caecal flora but it selectively increased *Bifidobacteri* and *Eubacteria* counts ($p<0.05$) and decreased the count of other microflora and concentrations of caecal phenols and indole ($p<0.0.5$). These results suggest that GTP and FOS in semi-purified diets can decrease mortality and change the caecal colonic flora population, but GTP shows antibiotic-like effects of non-selectively decreasing all colonic flora and then metabolites, and FOS acts selectively by increasing profitable microflora and decreasing production of caecal microflora metabolites besides volatile fatty acids. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 1 : 85-89)***

Key Words : Broilers, Green Tea Polyphenols, Fructo-oligosaccharides, Performance, Caecal Microflora Count, Caecal Microflora Metabolites

INTRODUCTION

Green tea polyphenols (GTP) are catechol and its metamer compounds extracted from green tea. It has been proved that GTP had antioxidation (Weisburger et al., 2001), deodorization (Terada et al., 1993), sterilization (Ryu, 1980; Hong et al., 2004) and pharmacological effects (Villiers et al., 1998). Effects of GTP on increasing *Lactobacilli* count and decreasing *Bacteroidaceae* count in chicken caecal contents (Terada et al., 1993) and porcine feces (Hara et al., 1995) have been reported.

It was reported that fructooligosaccharides (FOS) enhance the growth of *Lactobacillus* and *Bifidobacterium* spp., and possibly create a more favorable intestinal microbial environment in man (Hidaka et al., 1986; Mitsuoka et al., 1987) and chickens (Choi et al., 1994; Okumura et al., 1994; Oyarzabal and Conner, 1996).

If GTP and FOS could ameliorate the intestinal microenvironment, they might be able to change the quantity or the kinds of intestinal microflora, thus changing fermentation products i.e. volatile gases. If the output of volatile gases in the digestive tract was controlled, the excretion of faecal stink components would be reduced. On the other hand, if the tea polyphenols had the above bactericidal and pharmacological effects, they might be able to replace dietary antibiotics to some extent. If the GTP and the FOS had these assumed effects, their addition to feeds would have a role in reducing environmental pollution and in improving the quality of animal products.

Improvements in the quality of animal products by reduction of antibiotic residues and relief of environmental pollution by reduction of stink substance excretion have close relation with intestinal microflora.

The purpose of our experiments is to investigate the effects of GTP and FOS supplementation on performance and counts and metabolites of caecal microflora in female broiler chickens fed on semi-purified diets.

MATERIALS AND METHODS

Birds and management

With an average body weight of 1,152±15 g 4,500 female commercial broilers (Arbor Acre) were selected at 28 d of age from 10,000 birds fed on commercial feed (CP 200 g/kg, AME 13.39 MJ/kg) and housed in a semi-open henhouse. The selected birds were assigned randomly to control, GTP and FOS groups were housed in same semi-open henhouse which was disinfected adequately for a concrete floor, and supplied with wood shaving and an automatic feeder and water lines. Each group was averagely assigned to 6 treatments for 250 birds/treatment. Same feeding system as *ad libitum* was used on entire group during the experiment period, and the birds were slaughtered at 42 d of age.

Experimental diets

The control diet (Table 1) which contained 180.1 g crude protein/kg and 13.2 MJ AME/kg was formed from isolated soybean protein and maizestarch. Other

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Received March 17, 2004; Accepted July 26, 2004

Table 1. Composition of experimental diets (g/kg)

Ingredients	Group		
	control	GTP	FOS
Corn starch	683.2	679.1	679.1
ISP[1] (CP 823 g/kg)	218.8	218.8	218.8
Cellulose (980 g/kg)	35.0	35.0	35.0
Corn oil	20.2	20.0	20.0
$CaHPO_3$	19.0	19.0	19.0
$NaHCO_3$	10.0	10.0	10.0
GTP (972.2 g/kg)	-	4.1	-
FOS (980.2 g/kg)	-	-	4.1
Limestone	6.0	6.0	6.0
Choline chloride	2.0	2.0	2.0
DL methionine	1.3	1.3	1.3
Lysine	0.6	0.6	0.6
Cystine	0.9	0.9	0.9
Mineral mixture[2]	3.0	3.0	3.0
Vitamin mixture[3]	0.2	0.2	0.2
Calculated			
CP (g/kg)	180.1	180.1	180.1
AME (MJ/kg)	13.2	13.2	13.2
Calcium (g/kg)	79.8	79.8	79.8
Available phosphorus (g/kg)	40.0	40.0	40.0

[1] Isolated soybean protein.

[2] Supplies, as mg/kg of diet: Cu, 8.0; Fe, 80.0; Zn, 75.0; Se, 0.3; Mn, 100.0; I, 0.4.

[3] Supplies, as mg/kg of diet: vitamin A (all-trans-retinol), 4.2; cholecalciferol, 0.04; *d l*-alpha-tocopheryl acetate, 13; vitamin K_3, 3.3; folic acid, 2.0; pantothenic acid, 8.0; vitamin B_2, 3.0; vitamin B_1, 0.4; vitamin B_{12}, 0.006; biotin, 0.2.

experimental diets were prepared by mixing the GTP or the FOS into the control diet at the rate of 4.1 g/kg. The birds were supplied almost equal amounts of dietary CP and ME in all groups. The experimental diets were pelleted, crumbled and stockpiled in tanks.

Observations and samplings

Feed intake and bird mortality were noted before feeding at 08:30 h every day. At 42 d of age, the birds were weighed, and a random sample of 10 birds from each treatment were killed with CO_2 suffocation, and their cecum was removed, weighed and labeled. The cecum of 5 birds was stored promptly in liquid N_2 tanks to determine the microflora count. From the cecum of another 5 birds, the contents were removed and weighed in a clean room in preparation for the determination of the caecal microflora metabolites by methods described below.

Microflora incubation

Thawed caecal contents were placed in grinding tubes containing 9 ml anaerobic phosphate buffer solution (pH 6.8) at 4°C, in a CO_2 environment in a clean room. This liquid (10^{-1} dilution) was continuously diluted to10 times in series. Drops of the appropriate diluted liquid (0.5 ml) were spread on 1/3 to 1/4 of each medium area using a sterile Conradi-Stick and the media were then incubated in an anaerobic environment at 37°C for 2 to 3 days. Three non-selective and eleven selective media, as described by Mitsuoka et al. (1976), were used. Lecithinase-positive *Clostridium*, included within *Clostridium perfringens* were evaluated by the methods of Terada et al. (1994a).

The identification of bacterial groups was based on colony morphologies, Gram-reaction, spore formation and aerobic development.

The colony count in each medium was calculated after incubation and the logarithmic value was used to determine total bacterial count per gram of caecal contents. The total bacterial count was determined by totale each bacterial count.

Volatile gases

The composition of volatile fatty acids (VFA) was determined by the method of Koh et al. (1997): to 0.3 g of fresh caecal contents was added with 0.3 ml distilled water, then 0.2 ml, 40 mM crotonic acid standard solution. The mixed liquid was centrifuged at 10,000 rpm, for 10 minutes at 0°C. The proteins of molecular weight >10,000 were eliminated with a Mol Molecular Segregator from the upper liquid. The filtrate 1 μMol was used to determine the components of VFA by gas chromatography (Shimatsu, type GC-14C, Japan; column: GLScience, type FFAP20%-54, 2.0 m×3 mm, Japan) under column temperature 195°C and pressure of N_2 1.4 fkg/cm^2, H_2 1.5 fkg/cm^2 and air 1.0 fkg/cm^2.

Measurement of other metabolites was carried out by the method described by Yoshihara (1981) using gas chromatography (Shimatsu, type GC-14C, Japan; column: Shimadsu, type 17% Silicone SE-30, 2.0 m×3 mm, Japan) under column temperature 152°C and pressure of N_2 1.0 fkg/cm^2, H_2 1.5 fkg/cm^2 and air 1.0 fkg/cm^2.

The determined contents of metabolites were shown as mg/g caecal content.

Statistical analysis

Data were subjected to analysis of variance using SPSS Ver.11.0 (SPSS Institute, 2001) and means compared using the Tukey-Kramer method when the *F* test showed significance at $p<0.05$.

RESULTS

Production performance

Palpable effects (Table 2) obtained by the GTP and the FOS supplements were that the mortality rate was reduced significantly ($p<0.05$) for the two-week period. Body weight at 42 d of age and body weight gain, feed intake and feed intake: body weight gain from 28 to 42 d of age were not improved by the supplements.

Table 2. Effects of GTP and FOS supplement on female broilers performance from 28 to 42 d of age

	Group		
	Control	GTP	FOS
42 d of age Body weight (kg)	2.19±0.19	2.21±0.19	2.20±0.20
Body weight gain (kg)	1.04±0.0	1.06±0.08	1.05±0.09
Feed intake (kg)	1.92±0.15	1.92±0.15	1.92±0.13
Feed intake/body weight gain	1.84±0.09	1.81±0.07	1.83±0.07
Mortality (%)	3.23 ± 0.30^{b}	1.21 ± 0.11^{a}	1.86 ± 0.14^{a}

Means with different superscripts in the same row are significantly different ($p<0.05$).

Table 3. Effects of GTP and FOS supplement on caecal microflora counts of female broilers at 42 d of age (Log_{10} CFU/g)

	Group		
	Control	GTP	FOS
Bifidobacteria	8.42 ± 0.06^{b}	7.74 ± 0.03^{a}	9.14 ± 0.12^{c}
Bacteroidaceae	11.15 ± 0.18^{b}	8.99 ± 0.09^{a}	11.04 ± 0.10^{b}
Eubacteria	9.86 ± 0.20^{a}	9.01 ± 0.09^{a}	10.36 ± 0.12^{b}
Peptococcaceae	9.90 ± 0.16^{c}	8.26 ± 0.17^{a}	8.84 ± 0.13^{b}
*Clostridia**	4.33 ± 0.26^{b}	3.26 ± 0.11^{a}	3.02 ± 0.09^{a}
Lactobacilli	10.12 ± 0.16^{b}	9.34 ± 0.12^{a}	10.35 ± 0.31^{b}
Enterobacteriaceae	6.89±0.19	6.12±0.60	6.56±0.22
Streptococci	9.06 ± 0.13^{c}	7.33 ± 0.24^{b}	6.82 ± 0.22^{a}
Staphylococci	2.88 ± 0.04^{b}	1.67 ± 0.02^{a}	1.65 ± 0.26^{a}
Bacilli	7.34 ± 0.32^{c}	6.61 ± 0.10^{b}	5.27 ± 0.12^{a}
Summation	11.07 ± 0.12^{b}	9.30 ± 0.09^{a}	11.59 ± 0.13^{b}

* Lecithinase-positive. Means with different superscripts in the same rows are significantly different ($p<0.05$).

Table 4. Effects of GTP and FOS supplement on volatility gases content in caecal content of female broilers at 42 d of age (mg/g)

	Group		
	Control	GTP	FOS
Acetic acid	6.02 ± 1.03^{b}	4.04 ± 0.92^{a}	6.52 ± 0.25^{b}
Butyric acid	3.28 ± 0.21^{b}	1.34 ± 0.15^{a}	3.16 ± 0.33^{b}
Propionic acid	1.06 ± 0.16^{b}	0.14 ± 0.11^{a}	0.94 ± 0.30^{b}
Valeric acid	0.48 ± 0.11^{b}	0.22 ± 0.07^{a}	0.74 ± 0.05^{c}
Phenol	3.35 ± 0.02^{c}	1.49 ± 0.01^{a}	2.30 ± 0.03^{b}
Cresol	1.32 ± 0.01^{b}	0.77 ± 0.02^{a}	0.68 ± 0.01^{a}
Ethyl phenol	0.66 ± 0.01^{b}	0.37 ± 0.02^{a}	0.43 ± 0.02^{a}
Indole	0.11 ± 0.01^{c}	0.03 ± 0.01^{a}	0.07 ± 0.01^{b}
Summation	16.27 ± 0.73^{b}	8.41 ± 0.22^{a}	14.85 ± 0.19^{b}

Means with different superscripts in the same rows are significantly different ($p<0.05$).

Caecal microflora count

The GTP supplemented group had a significantly lower total bacteria count ($p<0.05$) than the other groups (Table 3). This was due to the counts of *Bifidobacteria*, *Bacteroidaceae*, *Peptococcaceae* and *Lactobacilli* in the GTP group being lower than those of other groups, the count of *Eubacteria* of the GTP group being lower than that of the FOS group, and the counts of Lecithinase-positive *Clostridia*, *Streptococci*, *Staphylococci* and *Bacilli* in the GTP group being lower than those of the control group. The FOS supplement gave significantly lower counts of *Peptococcaceae*, Lecithinase-positive *Clostridia*, *Streptococci*, *Staphylococci* and *Bacilli* than the control treatment ($p<0.05$), but significantly greater counts of *Bifidobacteria* and *Eubacteria* ($p<0.05$).

Caecal microflora metabolites

The concentrations (Table 4) of acetic acid, butyric acid, propionic acid, valeric acid, phenol and indole of the GTP group were significantly lower than those of the other groups ($p<0.05$), and cresol and ethyl phenol values were lower than those of the control group ($p<0.05$). The FOS supplement gave significantly lower concentrations of phenol, cresol, ethyl phenol and indole than those of the control group ($p<0.05$), but a significantly higher concentration of valeric acid ($p<0.05$).

DISCUSSION

The effect of FOS on bacteria has been found to be variable. Waldroup et al. (1993) found no effect but Choi et al. (1994) and Okumura et al. (1994) found the effect. Thereby the complicated feed ingredients may obscure the effect of FOS in the diet and affect the selective effect of FOS on the microflora. The present study was made with almost purified feed materials and was implemented in practical feeding conditions in order to objectively reflect the effects of GTP and FOS.

The present results indicate that the best effect of GTP and FOS supplements in semi-purified feed is to reduce mortality from 28 to 42 d of age (Table 2). In addition, the GTP can decrease significantly the caecal microflora count and their output of metabolites when compared to FOS, but the FOS can effectively increase the probiotics count (Table 3) and decrease the output of metabolites cecum other than VFA in the cecum (Table 4).

Since the GTP and the FOS did not contain protein and metabolisable energy and were present in a concentration as low as only 4.1 g/kg, they could not contribute directly to any improvement of feed utilization and performance in 14 days, even though they have the sterilization action (Ryu, 1980), decrease the *Bacteroidaceae* and increase the *Lactobacilli* counts (Terada et al., 1993; Hara et al., 1995), and modified the intestinal microenvironment.

The mortality in the two-week period of rapid growth (Table 2) was decreased significantly by the GTP and the FOS supplements. These additives therefore have the potential to reduce costs and increase meat yield by lower mortality, and also to reduce antibiotic residues because the medicaments were not used for all.

The major diseases that kill broiler chickens are sudden death syndrome, ascites syndrome and thigh syndrome.

Since these illnesses are popularly accompanied by excessive accumulation of abdominal fat (Giordani et al., 1994), some reports surmised that the cause of the death is higher growth rate (Leeson and Summers, 1997) and metabolic disturbances (Gonzales et al., 1998; Gonzalez et al., 2000). Because many studies have reported that the GTP have pharmacological effects (Villiers et al., 1998) such as improving low density lipoprotein (LDL) metabolism (Zhang et al., 1997; Yang et al., 2003), restraining peroxidation of LDL (Pearson et al., 1998; Yokozawa et al., 2002) and fat (Sano et al., 1995) and inhibiting liver estrone glucuronidation (Bao et al., 1998), in the present study the lower mortality observed with the GTP supplement might be related to the lipid metabolism and hormonal endocrine system affected by GTP.

Many studies have shown that FOS reduced serum cholesterol, LDL-cholesterol, and triacylglycerol in man (Mohammad, 2002) and rats (Oku, 1986; Tamura et al., 1997). Although a direct proof of the above effects is not found in birds, there may be similar effects. Mannanoligosaccharides as Bifidus factor as FOS can decrease abdominal fat deposition of broilers (Samarasinghe et al., 2003).

The FOS functioned probably on a different course from GTP, even if both had a lipid metabolism regulatory action. The main function of FOS has been reported to be that of improving the intestinal microenvironment and protecting systemic immunity by generating lactic acid as a fermentation product of *lactobacillus*, and selectively increasing or controlling some microbes counts (Chio et al., 1994; Okumura et al., 1994). Therefore, lower mortality observed in FOS supplement might originate in the known and conjectural reasons.

Different effects of GTP and FOS on the caecal microflora were definitely shown by the counts and classes (Table 3). The GTP indicated a bactericidal effect (Ryu, 1980) and it seemed to be nonselective in decreasing total counts and counts of all microflora except *Enterobacteriaceae* when compared with the control group. On the other hand, the FOS caused selective proliferation and inhibition of microflora; for example it changed the microflora structure where the resident *Bifidobacteria* and *Eubacteria* counts were significantly increased but *Peptococcaceae*, *Clostridia*, *Streptococci*, *Staphylococci* and *Bacilli* counts were decreased significantly. *Bifidobacteria*, *Eubacteria* and *Bacilli* were profitable animalcule (Monsan and Paul, 1995) and their main metabolites were lactic acid and acetic acid. Because spore *Bacilli* are nonresident and lives popularly in the inferior position, its count reduction might result in indirect influence from the FOS that preponderant lactobacillus and increased *Bifidobacteria* and *Eubacteria* inhibited their proliferation through competitive exclusion mechanism.

GTP showed sterilization-like effects, which resembled those of an antibiotic; on the other hand, FOS increased or controlled certain microbe alternatively (Table 4). Different influence fashions of GTP and FOS for microflora resulted in different caecal flora metabolites concentration (Table 5), for example, with compared to control group, concentration of volatile gases in caecal contents was reduced to 51% in GTP or to 56% in FOS respectively, however, used FOS did not reduce the concentration of total volatile gas, on the other hand, they reduced content of non-fatty acid which are phenol, ethylphenol, cresol and indole compared to control group.

Some investigators have reported that GTP (Terada et al., 1993) and FOS (Terada et al., 1994*b*) changed caecal flora counts, and reduced the concentrations of ammonia, phenol and cresol in caecal contents of chickens. The present study affirmed these actions of GTP and FOS and revealed that GTP could reduce concentrations of VFA, ethylphenol and indoles and FOS could reduce concentration of phenol, cresol, ethylphenol and indole in the caecum. The reduced VFA concentration suggested decreasing of effluvium substance excretion.

The results observed in the present study lasting two weeks were obtained in a situation in which birds experienced stresses such as feed changes, prohibition of veterinary drugs and ingestion of GTP and FOS. In this situation, intestinal changes in the microflora community might not have achieved equilibrium; hence it is necessary to investigate the effects of longtime supplementation and their mechanism.

CONCLUSIONS

Diets with 4 g/kg of GTP or FOS are able to reduce mortality of broilers 28-42 d-old. GTP decreases all microflora counts in the cecum and their metabolites contents through an antibiotics-like effect; FOS selectively promotes favorable microbes and inhibits microflora metabolites except volatile fatty acids in the cecum.

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