한국가금학회지 제 39권 제 4호, 253～260 (2012)
Korean J. Poult. Sci. Vol.39, No.4, 253～260 (2012)
http://dx.doi.org/10.5536/KJPS.2012.39.4.253

A Comparison of Feeding Multi-Probiotics and Fermented Ginseng Byproducts on Performance, Intestinal Microflora and Immunity of Broiler Chicks

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ABSTRACT This study was undertaken to investigate the influence of multi-probiotics, fermented ginseng byproducts and fermented sulfone as an alternative to probiotics on performance, intestinal microflora and immunity of broiler. A five weeks trial was conducted with one day old Ross×Ross broilers (n=340), divided into five groups which further divided into 4 replicates with 17 birds in each replicate. Birds were assigned to 5 dietary treatments as control, antibiotic avilamycin (AB), multi-probiotics (MP), fermented sulfone (FS) and fermented ginseng byproducts (FGB). Growth parameters were recorded on weekly basis while rest of the parameters viz. blood and faeces were collected at the end of the experiment. Growth parameters were not affected statistically by dietary treatments. However, numerically, higher body weight, splenocytes proliferation and lower total cholesterol and LDL values were found in MP treatment ($P>0.05$). S*almonella* spp. count ($P\leq0.001$) and *E. coli* ($P<0.001$) concentrations in the ileum were found lowest in AB treatment while FS group showed lowest level of yeast ($P<0.10$) and *Lactobacillus* spp. ($P\leq0.001$). Fecal ammonia and CO_2 emission was significantly decreased in FGB than other dietary treatments ($P<0.05$). It was concluded that multi-probiotics would be valuable feed additives to improve the growth performance, *Lactobacillus* proliferation and immunity of broiler chicks.

(Key words : multi-probiotic, fermented ginseng by-products, *Lactobacillus*, splenocyte, broiler)

INTRODUCTION

The use of antibiotics and other antimicrobials has been practiced for many years to enhance growth performance, disease prevention and efficient use of feeds in livestock feed industry (Barton, 2000). At present, a large number of natural growth promoters are commercially available including probiotics, prebiotics and immune modulators. These products have the potential to influence the intestinal tract in a positive way thus, improving the health, well-being and performance of animals (Fuller, 1989). Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the microbial flora and improve the general performances and prevent some specific intestinal pathogens (Waldroup et al., 2003). However the antibiotic growth promoters have been under scrutiny for many years and have been removed from the market in many countries (Ratcliff, 2000). The inclusion of antibiotics in the diets of animal has been prohibited in the EU countries since January 2006. In Korea, since July 2011, complete ban in the use of antibiotics as a growth promoter in animal feed has been come in to picture to ensure the safety of livestock products for consumers. Therefore, it is necessitated to find effective alternatives to antibiotic feed ingredients as prophylactic antibacterial and growth promoters. Feed additives such as enzymes, herbal products, microflora enhancers, immunomodulators, organic acids, probiotics, prebiotics or combinations of these products are being used as an alternative to antibiotics in poultry diet. Probiotics are being considered to fill this gap and already some farmers are using them (Trafalska and Grzybowska, 2004). There is sufficient evidence to show that probiotics are effective in enhancing the immune system, increasing body weight gain, reducing diarrhea and improving feed conversion efficiency (Patterson and Burholder, 2003). Several authors (Tortuero, 1973; Jin et al., 1998; Kalavathy et al., 2003; Mountzouris et al., 2007) found that probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a beneficial effect on broiler performance but limited infor-

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mation is available regarding the effect of multi-probiotics, fermented ginseng byproducts and sulfone as probiotics on broiler. Therefore, this study was undertaken to investigate the feeding of multi-probiotics, fermented ginseng byproducts and fermented sulfone on the performance, blood composition, intestinal microflora, noxious gas emission and immunity of broiler chicks.

MATERIALS AND METHODS

1. Birds and Dietary Treatments

Day old Ross×Ross broiler chicks (n=340) were obtained from a commercial hatchery and divided in to five groups. Each group had four replicates with 17 birds in each. Five corn-soybean based dietary treatments were formulated to meet Ross×Ross broiler nutrient requirements for starter (1 to 21 d) and finisher (22~35 d) period. Control or basal with crude protein (CP) 22 and 20% was provided for starter and finisher phases, respectively, and metabolizable energy was (ME) 3,100 kcal/kg. All diets were formulated to meet or exceed the nutrient requirements of broiler chickens (NRC, 1994) (Table 1). In 2nd, 3rd, 4th and 5th diet antibiotic avilamycin (AB), multi-probiotics (MP), fermented sulfone (FS) and fermented ginseng byproducts (FGB) was inoculated with 0.1% level to basal diet, respectively. The experimental duration was for 5 weeks. All the chicks were provided clean floor pen and rice hulls as litter. Throughout the experimental period the diet and water were supplied *ad libitum* to birds with 24 h access to lighting.

2. Preparation of Feed Additives

The probiotics products used in the experiment were supplied by TJ Bio Company, Republic of Korea. In the present experiment, avilamycin was used as an antibiotic (AB). Multi-probiotics (MP) was comprised of *Lactobacillus plantarum* (5×10^7 cfu/g), *Saccharomyces cerevisiae* (6×10^7 cfu/g) and *Bacillus subtillis*, (2×10^7 cfu/g). Fermented sulfur (FS) was prepared by fermenting mixed processed sulfur, gluten and *Bacillus subtillis* (2×10^7 cfu/g) at the room temperature. Same procedure was applied to prepare fermented ginseng byproducts (FGB) wherein processed sulphur was replaced by ginseng byproduct.

Table 1. Composition and nutrition analysis of experimental diets

Ingredients	Starter	Finisher
	(%)	
Corn	54.88	60.10
Soybean meal	32.60	30.88
Corn gluten meal	4.71	2.10
Soybean meal oil	4.00	3.70
Lime stone	1.32	1.44
Di calcium phosphate	1.73	1.14
Salt	0.40	0.40
L-Lysine	0.03	-
DL-Methionine	0.13	0.04
Vitamin premix[1]	0.10	0.10
Mineral premix[2]	0.10	0.10
Total	---------- 100.00 ----------	
Calculated value		
Metabolizable energy (kcal/kg)	3,100	3,100
Crude protein (%)	22.00	20.00
Lysine (%)	1.10	1.00
Methionine (%)	0.50	0.38
Ca (%)	1.00	0.90
Available phosphate (%)	0.45	0.35

[1]Contain per kg: vit. A, 12,000 IU; vit D_3, 5,000 IU; vit E, 50 mg; vit K_3, 3 mg; vit B_1, 2 mg; vit B_2, 6 mg; vit B_6, 4 mg; vit B_{12}, 25 mg; biotin, 0.15 mg; pantothenic acid, 20 mg; folic acid, 2 mg; nicotinic acid, 70 mg.
[2]Contain per kg: Fe, 66.72 mg; Cu, 41.70 mg; Mn, 83.40 mg; Zn, 66.72 mg; I, 0.834 mg; Se, 0.25 mg.

3. Data Collection

1) Performance Parameter

Body weight (g) of chickens in each pen was measured weekly to determine average body weight and weight gain. The feed intake (feed consumption, g) of birds per pen was recorded weekly and the feed conversion ratio of each pen was calculated [feed conversion ratio = feed intake (g)/net gain of body weight (g)].

2) Lipid Characteristic in Blood

At the end of feeding trail ten birds per treatment were kept for twenty four hours fasting. Blood samples were collected from the branchial vein and kept in a vacuum tube. Twelve hours after collection, the serum was separated and stored at −70℃ until analysis. The total cholesterol, triglyceride, HDL and LDL-cholesterol concentration in blood were measured by using test kit by enzymatic colorimetric method (AM 202-K; Asan Pharm Co., LTD, Korea).

3) Intestinal Microorganism

At 35 days of age, three broilers per treatment were killed and the carcasses were subsequently opened to collect the fecal sample from the ileum. One gram of collected sample was diluted with 10 mL of sterilized 9% saline solution (Joongwae Pharmaceutical Co., LTD, Korea), out of which 1 mL was transferred into 9 mL of the sterilized saline solution. Samples were serially diluted from 10^{-1} to 10^{-6}, and were injected by 100 μL in medium. *Salmonella, E coli,* yeast and *Lactobacillus* spp. were enumerated using SS agar, MacConkey agar, Yeast morphology agar and Rogosa agar of Difco (USA). Plates were then incubated at 37℃ for 24 h aerobically (SS, MacConkey and Yeast morphology agar) or 48 anaerobically (Rogosa agar) colonies so developed were counted and the result showed as colony-forming unit log10 per gram of sample.

4) Gas Emission of Feces

At the end of feeding trial, three broilers from each treatment were randomly selected and were allotted to individual cage. After three days adapting period, 50 g of feces were collected and kept in a 1,000 mL glass container and subsequently capped by parafilm. The containers were then stored for 5 min at room temperature. The NH_3 and CO_2 concentration were measured by using Gastec (GV-100, Japan).

5) Proliferation of Splenocyte

Three broilers per treatment at the age of 35 days were killed by severing the jugular vein and spleen was isolated. The acquired sample was kept separate and was dissociated between the frosted ends of two microscope slides. Erythrocytes were lysed in RBC lysis buffer for 5 min at room temperature and spun at 1,500 rpm for 5min. The splenocytes was washed two times with DMEM (Dulbecco's Modified Eagle Medium) and were cultured at a density of 10^6 cells/mL. The cells were cultured at 37℃ and 5% CO_2 in DMEM containing 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO) and antibiotics for 48 h. Cell proliferation was determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium MTS) assay (Buttke et al., 1993), using a Cell Titer 96 aqueous non-radioactive cell proliferation assay kit (Promega, Madison, WI). Absorbance was measured using a microplate reader at 490 nm.

6) Statistical Analysis

Analysis of variance (ANOVA) using GLM procedure of SAS (1998) was used to analyze the data. The difference among treatments was determined with Duncan's new multiple range test (Steel and Torrie, 1980) and significance was declared when the probability was less than 5% ($P<0.05$).

RESULTS AND DISCUSSION

In the present experiment no significant difference in broiler growth performance from 1 to 35 days of age was evident (Table 2), however numerically higher body weight and improved FCR was obtained from the broilers fed MP diets. The improvement of body weight gain of broiler fed MP might be due to the *Lactobacillus* spp. used in the supplement which colonize in the gastrointestinal tract and improve the digestibility of nutrients in the ileum (Pelicia et al., 2004), which agrees with the present findings. In addition, probiotics suppresses the growth of pathogenic microorganisms in the intestine and have potential to increase the bioavailability of dietary minerals resulting in an improved growth rate and feed efficiency (Toghyani et al., 2011). In contrast, with the present results, several workers (Watkins and Kratzer, 1984; Maiolino et al., 1992) reported that there were no significant differences in weight gain of chicken given diets with or without *Lactobacillus* cultures. Another group of researcher reported that the inoculation of probiotics has no effect on weight gains (Midilli et al., 2008; Alp et al., 1993) and feed consumption (Cavit, 2004; Yalcinkayal et al., 2008) but small improvements in efficiency (Mohan et al., 1996, Tortuero, 1973).

Table 2. Effect of feeding probiotics on the performance of broiler chicks

Item	Treatments						
	Control	AB	MP	FS	FGB	SEM	*P*-value
Weight gain (g)	1,639	1,744	1,766	1,720	1,708	22.80	0.53
Feed intake (g)	2,771	2,798	2,900	2,913	2,873	30.33	0.14
FCR	1.698	1.605	1.643	1.703	1.682	0.02	0.94
TCL (mg/dL)	97.14	110.42	98.85	103.85	102.00	2.94	0.67
TG (mg/dL)	18.14	21.85	17.71	22.43	25.85	1.11	0.12
HDL (mg/dL)	74.71	86.57	76.71	81.57	83.28	2.96	0.73
LDL (mg/dL)	18.80	19.48	18.57	17.80	13.54	1.00	0.36

SEM-Standard error of mean; AB, antibiotics; MP, mutiprobiotics; FS, fermented sulfone; FGB, fermented ginseng byproducts; FCR-feed conversion ratio; TCL-total cholesterol; TG-tri-glyceride ; HDL-high density lipoprotein; LDL-low density Lipoprotein.

Jin et al. (1996a) showed that an increase ($P<0.05$) in body weight gain and feed efficiency of broilers ($P<0.05$) when *B. subtilis* and *Lactobacilli* cultures were added to their diets. Kermanshahi and Rostami (2006) and Thitaram et al. (2005) reported that probiotics can improve the weight of birds. Thus, the variation of broilers performance to the microbial products might be due to the variation of microbial culture used in the probiotics, application level, feed composition, age and strain of the bird.

In present study, numerically lower level of triglyceride and LDL content was found in MP treatment but total cholesterol and HDL content was higher in AB and lower in control group (Table 2). It is noteworthy that a non significant reduction of serum cholesterol level (97.14 mg/dL) was found in control group. This value is similar to the mean value of 98.85 mg/dL cholesterol value obtained from MP supplemented group. The present finding is in line with (Djouvinov et al., 2005) who stated that the probiotic supplementation did not significantly affect the level of total cholesterol level in the broiler serum. In contrast, the addition of 0.05 and 0.1% *Lactobacillus* cultures to feed led to significant reduction of cholesterol levels in the serum of broilers (Jin et al., 1998). Similar depressing effect of probiotics on serum cholesterol concentrations has been found in broilers (Mohan et al., 1996). The decrease in cholesterol level could be due to cholesterol assimilation by the *Lactobacillus* cells (Gilliland et al., 1985), or to the co-precipitation of cholesterol with deconjugated bile salts (Klaver and Van der Meer, 1993) and reduction of pH in the intestinal tract, can be effective in reducing the cholesterol.

The composition of microflora in illeal intestine is presented in Table 3. There was significant difference ($P< 0.05$) in *Salmonella, E. coli* and *Lactobacillus* spp. populations due to dietary treatments. The illeal micro flora concentration of *Salmonella* and *E. coli* were found significantly ($P<0.05$) higher in control group. On the other hand, *Salmonella* concentration was remarkably ($P<0.05$) lower in antibiotic (AB) treatment, whereas MP, FS and FGB were intermediate and not different from each other. This might be due to the inoculation of *B. subtilis* culture in diet which favored the numbers of natural *Lactobacillus* and suppress *E. coli* in the gut wall. The *Lactobacillus* concentration in the ileum was also influenced ($P<0.05$) by the treatments where highest proliferation belongs to MP group whereas control and antibiotics were the moderate and FS showed the lowest proliferation. The *Lactobacillus* spp. used in the present study have a strong ability to attach to the intestinal epithelium of chicken, which are resistant to the bile and acidic conditions and are able to antagonize and competitively exclude some pathogenic bacteria *in vitro* (Jin et al., 1996b). In contrast, yeast population remained unaffected due to any dietary treatment; however a numerically higher value was obtained in control diet. Recently, Falaki et al. (2011) found *E. coli* have reduced the growth of poultry due to toxin production and

Table 3. Effect of feeding probiotics on ileal microbes of broiler chicks

Item	Treatments						
	Control	AB	MP	FS	FGB	SEM	*P*-value
Salmonella (log_{10} cfu/g)	5.18[a]	3.48[c]	4.41[b]	4.36[b]	4.56[b]	0.14	0.001
E. coli (log_{10} cfu/g)	6.99[a]	5.07[b]	5.17[b]	5.16[b]	5.35[b]	0.19	0.003
Yeast (log_{10} cfu/g)	6.24	4.94	5.91	4.90	5.85	0.21	0.07
Lactobacillus spp. (log_{10} cfu/g)	6.89[bc]	6.99[bc]	8.03[a]	6.65[c]	7.39[b]	0.13	0.001

[a-c] Value with a row with no common superscripts differ significantly ($P<0.05$). AB, antibiotics; MP, mutiprobiotics; FS, fermented sulfone; FGB, fermented ginseng byproducts.

utilization of nutrients essential to the host and suppression of microbes that synthesize vitamins and other host growth factor. In the ileum, populations of useful bacteria like *Lactobacillus* and *Bifidobacteria* (Ziggers, 2000) increases with the increase in pH of GIT which increases the production of volatile fatty acids. Therefore, the environment of GIT becomes unsuitable for the activity and proliferation of pathogens like *Salmonella*. In case of turkey, ingestion of *B. subtilis* culture had no significant effect on the *Lactobacillus* and *E. coli* populations in intestine (Saarchit and Sullivan, 1990). Other studies have also demonstrated the potential of probiotics to fortify the intestinal microflora of broiler chickens with beneficial bacteria and suppress potentially pathogenic bacteria (Koenen et al., 2004; Teo and Tan, 2007; Higgins et al., 2007).

Furthermore, fecal ammonia and CO_2 emission was significantly ($P<0.05$) decreased in MP, FGB and FS group (Table 4). Highest concentration of ammonia was found in control group which considerably reduced in FGB group whereas MP and FS group had an intermediated effect on NH_3 emission. Similarly, lowest ($P<0.05$) fecal CO_2 concentration was found in FGB treatment. The possible mechanism might be feeding of MP released digestive enzymes and thus increases nitrogen availability and nutrient digestibility in the intestine, the impact was reflected on the concentration of NH_3 and CO_2 in the feces (Yoon et al., 2004). In another study, it was mentioned that supplementation of probiotics significantly ($P<0.05$) decreased the fecal NH_3 and non-significantly reduced CO_2 gas emission in broiler, (Yoon et al., 2004). The present finding is in line with Lee et al. (2006) and Kim et al. (2003) reported that probiotics supplementation decreased the ammonia gas production in broiler.

A significantly higher ($P<0.05$) splenocytes proliferation was found in MP and control group (Fig. 1) which resulted in the increase in spleen lymphoid cell number. The enlargement of the spleen might be due to the microbial populations balance in the digestive tract. No significant difference was found among MP, control, AB and FGB group, however significantly ($P<0.05$) lower splenocyte proliferation was found in FS treated group. In meat-type strains, *Lactobacilli* did not influence the non-specific proliferative response of isolated spleen cells (Koenen et al., 2004) but immunoprobiotic *Lactobacilli* might enhance disease resistance which agrees the present findings. In another experiment, Clancy (2003) descri-

Table 4. Effect of feeding probiotics on noxious gas emission in broiler manure

Item	Treatments						
	Control	AB	MP	FS	FGB	SEM	*P*-value
NH_3 (ppm)	72[a]	52[ab]	37[bc]	29[bc]	14[c]	5.51	0.001
CO_2 (ppm)	1,733[a]	966[b]	933[b]	933[b]	767[b]	113.94	0.03

[a-c]Means in the same column for each parameter with different superscripts are significantly different ($P<0.05$).
AB, antibiotics; MP, mutiprobiotics; FS, fermented sulfone; FGB, fermented ginseng byproducts.

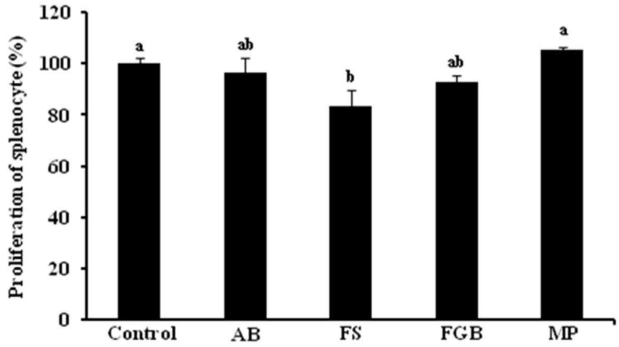

Fig. 1. Effect of feeding probiotics on proliferation of spleen in broiler. AB, antibiotics; MP, mutiprobiotics; FS, fermented sulfone; FGB, fermented ginseng byproducts.

bed that probiotic bacteria activate dendritic cells in Peyers' patches which in turn stimulate the T-lymphocytes. In this way these T-cells might also exert their immune modulation at distant mucosal sites. As more *Lactobacilli* reached the Peyers' patches they would activate more dendritic cells and indirectly increase the immune response.

CONCLUSION

In conclusion, multi-probiotics increased the body weight and decreases the triglyceride and LDL level in the blood. It also increases the concentrations of *Lactobacillus* and decreases the pathogenic organisms in the ileum. Higher proliferation of splenocyte was attained by feeding of multi-probiotics. Therefore, multi-probiotics would be valuable feed additives to improve the growth performance, lactobacillus proliferation and immunity of broiler chicks.

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(received: 2012. 8. 22, revised: 2012. 11. 22, accepted: 2012. 12. 3)