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Effects of Supplement with Different Level of *Bacillus coagulans* as Probiotics on Growth Performance and Intestinal Microflora Populations of Broiler Chickens

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Abstract: The aim of this study was to investigate the efficacy of *Bacillus coagulans* (*B. coagulans*), a spore-forming bacterium on performance and intestinal microflora of broiler chickens. A total of 192 one day old broilers were randomly assigned into four experimental treatments for 42 days. The experimental treatments received a corn-soybean basal diet and were as follows: basal diet (control), basal diet+0.005% *B. coagulans*, basal diet+0.02% *B. coagulans*, basal diet+0.04% *B. coagulans*. Each treatment had two replicates of 24 birds. Treatment effects on performance and intestinal microbiota composition of broiler were determined. Dietary supplementation of 0.005 and 0.04% *B. coagulans* significantly improved FCR (feed/gain) over the 21-42 days and the full 42 days. However, ADG was decreased by 0.005 and 0.04% *B. coagulans* supplementation over the 21-42 days and the full 42 days. In intestinal microflora, the count of Lactobacillus was significantly increased on duodenum and cecum by 0.02 and 0.04% *B. coagulans* diets treatments. Additionally, the count of *E. coli* was significantly decreased in duodenum and cecum on chicks fed with 0.02 and 0.04% *B. coagulans* diets. In conclusion, *B. coagulans* has the potential to be a beneficial microorganism on the modulation of intestinal microflora of broiler chickens.

Key words: *Bacillus coagulans*, probiotics, broiler, intestinal microflora, supplementation, experimental treatment

INTRODUCTION

The worldwide increase in the use of antibiotics as an integral part of the poultry and livestock production industry to treat and prevent infectious bacterial disease and as growth promoters at sub-therapeutic levels in feeds had generated the problem of the development of pathogenic bacteria antibiotic resistance during the past decades (Apata, 2009).

Due to the growing concerns of resistance bacterial can pass from animals to humans, the research for alternatives to replace in-feed antibiotics have gathered great importance in farm animal nutrition investigation in recent years. The effects of antibiotics in animal diets are highly related to the Gastrointestinal Tract (GIT) microflora (Bedford, 2000). GIT microflora has significant effect on host nutrition, health and growth performance by interacting with nutrient utilization and the development of gut eco-system (Barrow, 1992). High stocking density of battery farming systems may lead to an increase stress of chicks. The increase of stressors may depress immune function of broiler and therefore predisposes broilers to colonization of GIT by bacterial pathogens or other unfavorable microorganisms (Barnes, 1979; Hume *et al*., 2003). Probiotics are defined as viable micro-organisms used as feed additives which contributed to a beneficial effects for the host by improving GIT microbial balance or the properties of the indigenous microflora (Havenaar and Huis in't Veld, 1992; Fuller, 1989). A variety of microbial species have been used as probiotics including species of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Streptococcus* and a variety of yeast species (Simon *et al*., 2001). The effective probiotic products may provide a viable alternative to antibiotics use in broiler production. Probiotics have been widely used for improve growth performance of farm animals due to the reduction of pathogenic bacteria colonizing the GIT (Jin *et al*., 1998; Chambers and Lu, 2002; Hung *et al*., 2008; Lin *et al*., 2009). *B. coagulans*, a non-pathogenic, spore-forming, facultatively anaerobic, thermophilic, acid-tolerant, L^+ lactic acid producing strain which shows high resistance to heat and capability of preservation have been reported can improve growth performance of broiler

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(Cavazzoni *et al*., 1998). However, little is known about the effect of *B. coagulans* on GIT microflora population. Moreover, the optimal concentration for using *B. coagulans* as additive in broiler feed has not been established. Thus, the objectives of this study were to examine the performance and intestinal microflora population of broiler chickens fed with different doses of *B. coagulans*.

MATERIALS AND METHODS

Experimental design: The experimental protocol was approved by the Animal Ethics and Welfare Committee at the National Chiayi University (Taiwan) in accordance with the guide to the care and use of experimental animal by the Canadian Council on Animal Care (1993). This pilot study was conducted in a randomized complete block design with four diet treatments and two gender blocks. Total of 192 and 1 day old Arbor Acres broilers were randomly assigned into five treatment groups according gender of broiler. Each group was subject of a feeding treatment: basal diet without any additives (control), basal diet+0.005%, basal diet+0.02% *B. coagulans*, basal diet+0.04% *B. coagulans*. The count of microflora of *B. coagulans* powder was 8 $\log_{10}$ CFU^{-g}. There were 24 chicks per replicate and two replicates per treatment group. The feed and water were supplied *ad libitum* and illumination was 24 h florescent lighting throughout the entire experimental period. The diet was formulated to meet the nutrient requirements of the broiler that during the growing phase (0-21 day) and finishing phase (21-42 day) according to the NRC (1994) (Table 1). Temperature and humidity of the surrounding environment were recorded daily. Feed intake and body weight were recorded weekly. At end of experiment, four birds were randomly selected from each pen and sacrificed by bloodletting through the carotid artery for GIT digesta collection.

Intestinal microflora analysis: Intestinal digesta samples were obtained by massaging the tract from the gizzard, duodenum and cecum. The obtained intestinal digesta samples were then serially diluted and plated in count agar. Aerobic and anaerobic microorganisms were cultured in count agar plate (Merck, 1.05463). Lactobacillus strain was cultured in MRS-agar (Merck, 1.1066). *E. coli* was cultured in coliform Agar (MERCK 1.10426). Aerobes and *E. coli* medium plates were placed in an incubator at 37°C for 48 h. Anaerobes and Lactobacillus medium plates were placed in an anaerobic jar (Merck 1.16387) with an anaerobic gas pack system (Anaerocult® MERCK 1.13829) at 37°C for 48 h. The microflora enumerations were expressed as $\log_{10}$ Colony Forming Units (CFU) per gram.

Table 1: Formula and calculated composition of experimental diets

Ingredients	Day 0-21	Day 21-42
Percentage		
Corn, Yellow	42.00	51.45
Soybean meal (43.5%)	20.00	20.00
Dehulled soybean meal	25.60	16.50
Yeast powder (40%)	2.50	2.50
Molasses	3.00	3.00
Soybean oil	3.00	3.00
Salt (Iodized)	0.30	0.30
Dicalcium phosphate	1.30	1.20
Limestone (Pulverized)	1.60	1.40
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.15	0.15
Choline chloride (50%)	0.10	0.10
Betaine	0.10	0.10
DL-Methionine	0.20	0.15
Total	100.00	100.00
Calculated value		
Crude protein (%)	23.00	20.00
ME (kcal kg^{-1})	3133.00	3077.00
Calcium (%)	1.00	0.90
Available phosphorus (%)	0.41	0.38

[1]Vitamin premix (content per kg): vitamin A, 12,500 IU; vitamin D_3, 2,500 IU; vitamin E, 20 IU; vitamin K_3, 2,500, mg; vitamin B_1, 2,000 mg; vitamin B_2, 5,000 mg, vitamin B_6, 3,000 mg; vitamin B_{12}, 12 mg; niacin, 35,000 mg; pantothenic acid, 12,000 mg; folic acid, 1,000 mg. [2]Mineral premix (content per kg): Fe, 70 g; Zn, 90 g; Cu, 10 g; Mn, 80 g; Se, 15 g; I, 0.4 g

Statistical analysis: Data on growth performance (ADG, ADFI and FCR) were based on mean of all birds from each pen whereas data on intestinal microflora were based on mean of broilers selected from each pen. Experimental data were analyzed using ANOVA procedure for a randomized complete block design under the following model:

$$Y_{ij} = \mu + D_i + G_j + e_{ij}$$

Where:

Y_{ij} = The dependent variable
μ = General mean
D = Diet effect (i = 1-4)
G_j = Block by the gender of birds (j = 1-2)
e_{ij} = Experimental error

The Tukey's honest significant difference was used to test the differences between treatment means. The statistical analysis was done using the SAS program (SAS Institute Inc., USA). Differences among means with $p<0.05$ were accepted as representing statistically significant difference.

RESULTS AND DISCUSSION

Growth performance: Chicks were in general health over the 42 days experimental period. The effects of *B. coagulans* administration on broiler growth performance are shown in Table 2. The supplementation of *B. coagulans* did not exhibited positive effect on ADG of broiler. ADG was decreased by 0.005% *B. coagulans* and 0.04% *B. coagulans* supplementation over the 21-42 day

Table 2: Effect of dietary *Bacillus coagulans* supplementation on growth performance of broilers [1, 2]

	Treatment				
Item[3]	Control	0.005% BC	0.02% BC	0.04% BC	SEM
ADG					
0-21	37.30	36.20	37.20	36.30	0.11
21-42	67.20[a]	65.30[b]	67.50[a]	66.00[b]	0.13
0-42	52.30[a]	50.80[b]	52.40[a]	51.20[b]	0.16
ADIF					
0-21	52.70	50.50	50.50	50.40	0.17
21-42	116.70[a]	110.30[c]	114.20[ab]	112.00[bc]	0.16
0-42	84.70[a]	80.20[b]	82.50[b]	81.20[b]	0.18
FCR					
0-21	1.41	1.40	1.36	1.39	0.11
21-42	1.74[a]	1.73[a]	1.69[b]	1.75[a]	0.06
0-42	1.62[a]	1.61[a]	1.57[b]	1.62[a]	0.07

[1]ADG means Average Daily weight gain (g day^{-1}); ADFI means Average Daily Feed Intake (g day^{-1}); FCR means Feed Conversion Rate (feed/gain). [2]BC means *Bacillus coagulans*. [3]No linear effect was found in different doses of *B. coagulans* on growth performance ($p>0.10$). [a-c]Means within the same row with different superscripts differ significantly ($p<0.05$)

and the full 42 days ($p<0.05$). Moreover, ADFI was decreased in broiler fed with *B. coagulans* over the full 42 days ($p<0.05$). Conversely, dietary supplementation of 0.005 and 0.04% *B. coagulans* significantly improved FCR over the 21-42 day and the full 42 days ($p<0.05$).

Intestinal microflora: In the present study, the conventional microbiological techniques using selective agar media were used to analyze the microflora enumeration of *B. coagulans* product and intestinal digesta samples. The effects of dietary supplementation of *B. coagulans* on intestinal microflora are shown in Table 3. The count of Lactobacillus was significantly increased on duodenum and cecum by 0.02 and 0.04% *B. coagulans* diets treatments ($p<0.05$). Moreover, the count of *E. coli* was significantly decreased in duodenum and cecum on chicks fed with 0.02 and 0.04% *B. coagulans* diet ($p<0.05$). There was a linear effect on increased number of Lactobacillus in both duodenum and cecum by dietary supplementation with 0, 0.005, 0.02 and 0.04% *B. coagulans* ($p<0.05$). Similarly, a linear decrease of number of *E. coli* was found in *B. coagulans* administration on duodenum and cecum ($p<0.05$).

In this study, the spore-forming bacterium *B. coagulans* was added directly to the chick's diet without fermented procedure in order to investigative the probiotic ability of *B. coagulans* as an alternative to antibiotics with growth promoting and balance of GIT eco-system. No linear effect was found in different doses of *B. coagulans* on growth performance. FCR was improved by 0.005 and 0.04% *B. coagulans* supplementation although, there were no positive effects of ADG and ADFI. Previous studies reported that dietary supplementation of probiotics can improve FCR of broiler (Jin *et al*., 1998; Lin *et al*., 2009). However, others studies reported that probiotics have no differences in FCR between probiotics treatment and control treatment (Watkins and Kratzer, 1983, 1984; Huang *et al*., 2004).

Table 3: Effect of dietary *Bacillus coagulans* supplementation on intestinal microflora of broilers [1, 2]

	Treatment					
Items	Control	0.005%	0.02%	0.04%	SEM	Linear
	----------$\log_{10}$ CFU^{-g}----------					
Duodenum						
Lactobacillus	6.36[c]	6.58[c]	7.12[b]	7.56[a]	0.22	*
E. coli	6.59[a]	6.58[a]	6.43[b]	6.40[b]	0.15	*
Cecum						
Lactobacillus	8.40[c]	8.62[b]	8.90[a]	9.01[a]	0.16	*
E. coli	8.74[a]	8.39[b]	8.39[b]	8.38[b]	0.11	*

[1]CFU means Colony Forming Unit. [2]BC means *Bacillus coagulans*. [a-c]Means in the same row with different superscripts are significantly different ($p<0.05$). *means significantly difference of linear effect among different doses of *Bacillus coagulans* supplementation

These inconsistent results may be due to differences in the probiotic bacterial strain and the ability of probiotics on modulation of intestinal microflora. The previous study showed that the improvement of growth performance is highly related with the number of *E. coli* in GIT.

The administration of probiotics results in the modulation of gut micro-ecology conditions by suppresses harmful microorganisms (Line *et al*., 1998; Pascual *et al*., 1999) and favor beneficial bacterial. In this study, dietary supplemented with *B. coagulans* contributed to the modulation of GIT microflora balance as evidenced by significant increases in the enumeration of Lactobacillus and decrease in the enumeration of *E. coli*. Yu *et al*. (2007) indicated that dietary probiotics supplementation enhance the Lactobacillus counts in the crop, ileum and cecum of broilers. An early study by Dharmawan *et al*. (2006) reported that the lactic acid bacteria supplementation could inhibit the adhesion of *E. coli* O157:H7 in intestine of human. Gonzalez *et al*. (1995) used a mixture *Lactobacillus* species as bacteriotherapy against infantile diarrhea caused by *E. coli* and Salmonella. Although, the stressful physiological and environmental conditions often promote the proliferation of pathogens in the digestive tract of chicks incur reduction of daily weight gain. Dietary supplement with probiotics can potentially alter gut microflora by selectively stimulating the growth of beneficial bacteria while suppress the growth of pathogenic bacteria (Van Heugten *et al*., 2003). In this study, 0.02 and 0.04% *B. coagulans* diet can enhanced Lactobacillus number and reduce *E. coli* in both duodenum and cecum indicated that dietary supplement with more than 0.02% *B. coagulans* have efficacy to improve broiler intestinal microflora balance.

The objective of this study was to investigate the efficacy of *B. coagulans* on broiler. It was hypothesized that broilers fed with *B. coagulans* diet would have better performance and a balance intestinal microflora. Previous research has shown that the supplementation of *B. coagulans* as probiotics to the broiler diet significantly improves chickens performance compared with chickens

fed with diet without extra additive or with antibiotics as growth-promoting prophylactic additive (Cavazzoni *et al*., 1998). Results in the current trail revealed that dietary supplementation of *B. coagulans* on can improve FCR of broiler. In addition, the administration of *B. coagulans* can modulated the composition of intestinal microflora by increased the count of Lactobacillus and decreased count of *E. coli.*

CONCLUSION

In conclusion, *B. coagulans* may be proposed as a substitute for antibiotics and recommended as additive in broiler diet. The range between 0.02 and 0.04% may be the optimal dose for supplement *B. coagulans* in broiler diet.

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