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Effects of *Bacillus subtilis* on Growth Performance and Resistance to Salmonella Infection in Broiler Chickens

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ABSTRACT The experiment was undertaken to see the effects of *Bacillus* sp. on the growth performance and disease resistance to *Salmonella* sp. infections. The use of probiotic microbes in poultry is commonly practiced. In this study, *Bacillus subtilis* was tested using a total of 120 chicks of age of 1 day after hatching. The growth traits examined were body weight gain and feed conversion rate. And also, the Salmonella resistance of *Bacillus subtilis* was tested after the chicks were orally administered with *Salmonella pullorum* by gavage force injections. The result showed that *Bacillus subtilis* yielded a high feed efficiency, consequently increased growth rate. For the effect of *Bacillus subtilis* on Salmonella infection, *Bacillus subtilis* significantly improved the resistance to *Salmonella pullorum* infection. Various clinical symptoms of *Salmonella* infection were highly decreased by addition of *Bacillus sp*.

(Key words : *Bacillus subtilis*, broiler chicks, Salmonella resistance, body weight gain, feed conversion ratio)

INTRODUCTION

Many studies have been made on the efficiency of microorganisms as feed additives for the various economic traits of poultry (Kim et al., 2013; Lee et al., 2013; Hwangbo et al., 2011; Kim et al., 2010; Jung et al., 2010; Kim et al., 2008). The microbes being widely used in poultry industry are termed as probiotic microbes such as *Lactobacillus* sp, *Bacillus* sp, yeasts and etc. The main concept of using microbes in poultry farms is the extra enhancement of economic traits after being added to the main diets. The main functions of microbes in chickens as well as other livestock species are the metabolites that are excreted by the microbes, especially the various types of enzymes and other anti-bacterial and anti-fungal excretes. The cell count of the microbes is less emphasized than the metabolites from the microbes. Out of several genus of *Bacillus* species, *Bacillus subtilis* is most commonly used as feed additives in the probiotics industry of livestock. *Lactobacillus* species are also commonly used as feed additives. Some toxins excreted by the microbes often can be useful or harmful, depending upon the metabolites produced by the microbes. For green farming of environment-friendly objectives, microbes as probiotic microorganism are the very useful alternative over conventional farming with some use of chemical and artificial materials (Vila et al, 2009) and also stated that the well-balanced native microbes can decrease the presence of Salmonella in optimal conditions. However, these conditions are also influenced by various environmental factors. As reported by Awad et al. (2006), Salmonella is the major pathogen for the poultry industry. Both growth performance and meat quality were improved by the probiotic microbes in chickens (Lee et al., 2013). Similar trend was found in layers (Jung et al., 2010).

MATERIALS AND METHODS

1) Experiment

The chicks were 1 day old broiler chicks of commercial lines. A total of 120 chicks were used in the experiment. They were all measured for their weights individually. The microbe used in this study is the *Bacillus subtilis* which was isolated from the soil around Suwon, Kyonngi-Do Korea. Hereafter the term of *Bacillus* sp refers *Bacillus subtilis* throughout the paper. They were assigned randomly into four groups; (−) Control (=T1), (+) Control (=T2), and the two *Bacillus subtilis*-added treatment groups in that one without *Salmonella* infection (=T3) and the other with induction of *Salmonella* (=T4) after 14 days feeding since hatching. The microbes used were *Bacillus subtilis*. For a feeding trial, they

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were fed ad libitum with a commercial starter (the formula shown in Table 1) for 2 weeks and then, the treatment group was orally infected with 1 mL of PBS suspension containing 1 × 10^8 cfu *Salmonella pullorum* by gavage force feeding. The experimental design with 4 levels of the treatment is descri- bed in Table 2. During the experiment, measured were body weight gain (BWG), total feed intake at day 1, 7, 14 and 21. After *Salmonella pullorum* was induced, clinical symptoms such as mortality and mobility were observed. And also, autopsy was practiced after 3 weeks old. The cultivated liquid broth of *Bacillus subtilis* was dried with glucose powder. The *Bacillus subtilis* powder then was added to the feed as 0.3 % of the total feed.

2) Statistical Model

The analysis made was the one way ANOVA using SAS. The model analyzed was as;

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where y_{ij} = the observed data observation

μ = overall mean

T_i = Treatment group (1 to 4)

e_{ij} = residual

The mean differences among the treatment groups were tested by Tukey's method.

RESULTS AND DISCUSSION

1) *Growth Performance*

The commercial broilers have been bred for meat consumption with fast growth and high feed efficiency. The body weight gain and feed intake in each treatment group were calculated, which was shown in Table 3. For the effect of Salmonella infection vs. non-infection (T1 vs. T2), the difference was about 75 g in body weight gain at day 21 (3 weeks of age). For the effect of Salmonella infection on growth performance, when the chicks were infected (T2) and the infected group added with *Bacillus subtilis* (T4), the body weight gains were 360.33 g and 427.69 g, respectively. The increase of growth performance was also reported by Kim et al. (2008), Hwangbo et al. (2011) and Kim et al. (2010). The addition of *Bacillus subtilis* was phenomenal such that even when the chicks were infected, the addition of *Bacillus subtilis* significantly compensated the loss of body weight gain and

Table 1. Broiler feed formulation

Raw material	Percent (%)
Ground corn	44.8
Rice barn	10
Soy bean meal(44% protein)	31.2
Fish meal(60% protein)	8
Oyster shell meal	0.6
Di calcium phosphate	0.6
Vegetable oil	4
DL-Methionine	0.2
Salt	0.35
Multi-mineral and multi-vitamin	0.25
Total	100
% total feed protein	23
Net consume energy(kg.cal/kg)	3,150

Table 2. Treatment group used in the experimental design

Treatment[1)]	*Bacillus subtilis* added in feed	*Salmonella pullorum* induced orally
T1	No	No
T2	No	Yes
T3	Yes	No
T4	Yes	Yes

[1)] T1 : negative control, T2 : positive control, T3 : negative control added with *Bacillus* sp., T4 : positive control added with *Bacillus* sp.

Table 3. Growth rate and feed efficiency for 3 weeks old broiler

Treatment[1)]	Body weight gain; BWG (g/bird)[2)]	Feed intake (g/bird)	Feed conversion ratio; FCR
T1	435.6 ± 16.83^a	611.5 ± 16.93^a	1.40
T2	360.33 ± 19.53^b	568.33 ± 14.01^b	1.58
T3	557.17 ± 16.71^c	625.63 ± 17.52^a	1.12
T4	427.69 ± 28.91^a	543.0 ± 16.81^b	1.27

[1)] T1: negative control, T2: positive control, T3: negative control added with *Bacillus* sp., T4: positive control added with *Bacillus* sp., Note: Different superscripts in columns significantly differ (p<0.05).

Fig. 1. Salmonellosis symptom(Cloacae prolapsed), left (not infected) and right(infected).

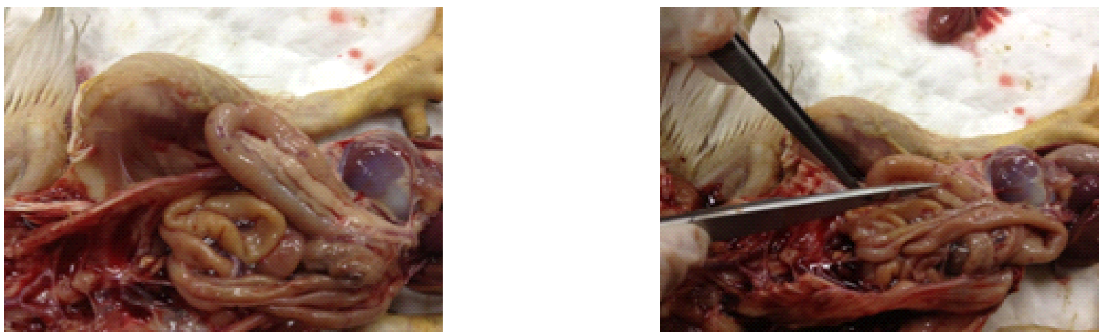

Fig. 2. Enteritis of anterior of small intestine of *Salmonella* infection.

the difference between them (T2 vs T4) was significantly different, which is possibly due to one of the potential immunological enhancement of the infected chicks. Therefore, the addition of *Bacillus subtilis* could reduce the risk of body weight gain loss even when the chicks were under *Salmonella* infection. Consequently, feed conversion ratio (FCR) was significantly lower when *Bacillus subtilis* was added as a feed additive, which is of major concern for broiler farmers in sense of farm profits. Hume (2011) reported the probiotic function in broiler chicken that induce intestinal villi absorption efficiency would lead to raise up the feed utilization of chicken and induce the immunity of intestinal tract. In addition with improved body weight gain and FCR, the clinical symptoms were also investigated and shown in Table 4 and Fig. 1 and 2.

2) Salmonella Resistance

Different types of typical clinical symptoms of Salmonellosis were described in Table 5 and pictured in Fig. 1 and 2. The chicks with feeding *Bacillus subtilis* showed significantly high resistance to *Salmonella* infection (gavage force feeding through oral) when compared with the groups with no addition of *Bacillus subtilis*. Even for the groups without addition of *Bacillus subtilis*, the mobility and mortality rates were highly significant in that mobility rate was improved by 80% and by 43% for the mortality of the infected groups (T1 vs. T2) as shown in Table 4. When the chicks were infected with *Salmonella*, the mobility and mortality rates were also drastically decreased by 50%. When infected with *Salmonella sp.* the clinical signs were shown in Fig. 1 and 2. The typical symptoms of Salmonellosis were pictured in Fig. 1 and 2. The Salmonellosis symptom in Fig. 1 indicated the sign of Cloacae prolapsed and Fig. 2 indicated the enteritis of ante-

Table 4. The Salmonella susceptibility rate in each treatment

Treatment[1)]	Salmonella susceptibility	
	Mobility rate (%)	Mortality rate (%)
T1	0	0
T2	24 (80)	13 (43.33)
T3	0	0
T4	14 (46.67)	6 (20)

[1)] T1: negative control, T2: positive control, T3: negative control added with *Bacillus* sp., T4: positive control added with *Bacillus* sp.

Table 5. The clinical symptom of each treatment after Salmonella challenge

Treatment[1)]	The number of chicken with clinical symptom of Salmonellosis (*Salmonella pullorum*)				
	Weakness & dejection	Reluctance to move	Anorexia	Yellowish diarrhea	Cloacae prolapsed
T1	0	0	0	0	0
T2	24	10	24	4	8
T3	0	0	0	0	0
T4	10	2	9	0	1

[1)] T1: negative control, T2: positive control, T3: negative control added with *Bacillus* sp., T4: positive control added with *Bacillus* sp.

infection. To summarize the conclusion of this study, the trial of broiler chicks was undertaken to see the effects of *Bacillus subtilis* on growth traits and Salmonella resistance. The growth performance of broiler chicks after adding *Bacillus subtilis* to the feed was significantly improved and the Salmonella resistance was also highly increased. The mobility and mortality rates were considerably improved and thus, some strains of *Bacillus subtilis* could be an excellent feed additive as well as an alternative of chemical vaccination or treatment for the pathogenic diseases of chickens. Out of various sources of probiotic microbes, certain microbes such as the *Bacillus subtilis used in this study showed significant impacts on the performance of broiler chicks. Of importance is not only the* cell count of the microbes but more importantly, the metabolites excreted from the microbes are the better criteria for the choice of the probiotic microbes.

Table 6. The gross lesion of each treatment after Salmonella challenge

Treatment[1)]	The gross lesion of Salmonellosis (*Salmonella pullorum*)		
	Enteritis of anterior small intestine	Engorgement of kidneys and spleen	Bronzed enlarged liver
T1	0	0	0
T2	20	0	0
T3	0	0	0
T4	10	0	0

[1)] T1: negative control, T2: positive control, T3: negative control added with *Bacillus* sp., T4: positive control added with *Bacillus* sp.

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