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Effect of Probiotic on Microflora Population and Carcass Yield of Quail, *Coturnix japonica*

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ABSTRACT

This study was conducted to determine the effect of dietary probiotic on intestinal and fecal microflora and carcass yield of broiler quails during the four weeks of feeding period. The quails were fed a basic diet (group 1) which acted as the control group; another 3 groups were fed basal diet with probiotic added at concentrations of 0.05%, 0.20% and 0.35% respectively for 28 days after two weeks of brooding. On day 42, fecal sample and intestinal sample were collected for microbial analysis and carcass yield of the quails was evaluated by cutting the selected parts of the carcass. The results showed that there was a significant difference ($p < 0.05$) in *Escherichia coli* and *Lactobacillus* population in quail's feces between control and treatment group. Otherwise, there was no significant difference ($p > 0.05$) in *Escherichia coli* population in quail's intestine between control and treatment group, but significantly ($p < 0.05$) different in *Lactobacillus* population. Different inclusion rate of probiotic including control showed negative result of *Salmonella* in both intestine and feces of quail. In addition, supplementation of the diet with probiotic resulted in higher dressing and legs percentage of the carcass but do not affect the breast percentage. This shows that adding probiotic to the broiler quail's diet can improve the performance of the quails by increasing the beneficial microflora and reducing the pathogenic microorganisms, and relatively higher carcass yields.

Keywords: Probiotic, *Coturnix japonica*, intestinal microflora, carcass quality, *Bacillus subtilis*

INTRODUCTION

Nowadays quail production contributes to fast developing sector of poultry industry. The demand on the quail products especially meat is currently increasing, thus becoming consumer's second choice after chicken, especially in developing countries (Jeke et al., 2018a). This is because quail meat provides more nutritional value than chicken meat as it has low skin fat and low cholesterol value (Hubrecht and Kirkwood, 2010). Quail meat has excellent taste and texture rather than chicken meat (Bakoji et al., 2013). Their meat contains lots of

micronutrient with wide range of vitamins including B complex, folate, and vitamin E and K. Quails are believed to provide many advantages to human health due to their low cholesterol value in meat and rich in HDL cholesterol in egg (Imchen, 2013). Quail farming has the particular advantage of encouraging the growing market demand for poultry products. This is because quail does not only provide animal protein in the form of meat and eggs, but also provides a source of income (Jeke et al., 2018b). Quails can be marketed by six weeks since they have short interval of rearing which is six to seven cycles per year (Saharani, 2010). Thus, there will be a great opportunity in quail farming in terms of economy to meet the demand.

In quail farming, good quality feed is necessary to obtain rapid growth for their performance. However, better feed alone may not serve the optimum growth performance of the quail. Antibiotics have been widely used for the past decades throughout the globe as growth promoting agents in animal feeds, mainly in the areas of poultry and pig farming (Denli et al., 2004; Pambuka et al., 2014). However, threats to the use of antibiotics that promote growth is rising as people fear their usage in animal feed can cause the growth of antibiotic-resistant bacteria that are dangerous to humans (Denli et al., 2004; Widiyanto & Indrawan, 2018). Recently, inclusion of probiotics to poultry diet has been found to improve growth performance and feed conversion ratio of different domesticated poultry (Ayasan et al., 2006; Haryati, 2011; Olnood et al., 2015; Widiyanto & Indrawan, 2018). The initial concept of probiotics is to modify the nature of poultry intestine by administering live microorganism that could benefit the host bird (Ouwehand et al., 2002; Alkhalf et al., 2010). Using probiotic can improve product and performance of poultry by enhancing appetite, intestinal balance, digestive enzyme, synthesizing vitamins, reduce pathogenic microbial colonization improve absorption of nutrients and decrease cholesterol level (Khan et al., 2011). Therefore, by administering probiotic in their feed may help to utilize a better growth performance and increase the production of quail.

The aim of the current study was to determine the effect of probiotics on microflora population in intestine and feces of broiler quails and to compare the carcass yield of broiler quails with and without probiotics.

MATERIALS AND METHODS

Study site

The study was conducted at Faculty of Bioresource and Food Industry (FBIM), Universiti Sultan Zainal Abidin, Besut Campus. The place is selected because of it suitability to rearing 240 broiler quails starting from day old chick (DOC) with brooding temperature of 35 °C and decreased after 1 week by 3.5 °C until 42 days. All the activities including the laboratory works were carried out at this FBIM cages and laboratories until the end of the study.

Experimental design and treatment

A total of 240 broiler quails from commercial breeding were randomly distributed into four groups, with 60 broiler quails per group. Each group was divided into three replicates with 20 quails each. The quails were fed with commercial diet and water ad libitum throughout the study. The first group as the control (without inclusion of probiotic) was fed basal diet. The other groups were fed the basal ration supplemented with probiotic of 0.05%, 0.20%, and 0.35% respectively. The trial period was 42 days.

Enumeration of intestinal microflora

After day 42, the enumeration of intestinal microflora population was conducted. Twenty broiler quails were randomly selected from each replicate and sacrificed by severing the jugular vein. About 1 g of samples was put into sterilized tubes and diluted into sterilized normal water to 1:10. The mixture was serially diluted up to 7. Using a micropipette, 0.1 ml dilution was pipetted and plated on agar plates using L shape glass rod, and was

incubated before counting the colonies on plates. *Escherichia coli* counts were performed on Eosin Methylene Blue (EMB) agar and incubated for 24 hours at 37 °C. Xylose Lysine Deoxycholate (XLD) agar was used for culturing *Salmonella* and incubated for 24 hours at 37 °C (Gao et al., 2017). *Lactobacilli* was performed on deMan, Ragosa and Sharpe (MRS) agar for 48 hours incubation at 37°C under anaerobic condition (Shokryazdan et al., 2017). Duplicates were made for each dilution and each medium used. Petri dishes with 30 to 300 colonies were counted using a colony counter (Jin et al., 1996).)

Enumeration of fecal microflora and observation

At day 42, fecal samples from each treatment were collected. A portion (1 gram) of feces was serially mixed with 9 ml of distilled water. 0.1 ml of the dilution was then pipetted and placed on agar plates by using L shape glass rod. *Lactobacilli* was enumerated on MRS agar and incubated at 37°C for 48 hours under anaerobic condition. *Salmonella* and *Escherichia coli* were enumerated on XLD and EMB agar respectively and incubated at 37°C for 24 hours. Each dilution and medium used was duplicated. All plates were observed, and the colonies were counted by using Colony Counter and the data was recorded.

Calculation

The number of colonies were counted by using the formula below:

$$\mathrm{N(CFU/ml)} = \frac{\mathrm{c\ x\ d}}{\mathrm{v(n1) + v(n2)}}$$

N: number of colonies
c: sum of colonies on all plates counted
v: volume applied on each plate (ml)
n1: no. of plate counted at the 1st dilution
n2: no. of plate counted at 2nd dilution
d: dilution from which 1st counted start

Isolation of Bacteria

Eosin Methylene Blue (EMB) agar was used as selective media for culturing the *E. coli* bacteria. The preparation of EMB agar was conducted by mixing 36 g of EMB medium with 1 L of distilled water. The mixture was boiled for one minute until the medium completely dissolved. The mixture was sterilized by autoclaving at 121 **°C** for 15 minutes. Xylose Lysine Deoxycholate (XLD) agar was used as selective media for culturing the *Salmonella* bacteria. The preparation of XLD agar was conducted by suspending 55 g of XLD medium in 1 L of distilled water. The medium was heated with frequent agitation until the medium boils. The medium was transferred immediately to a water bath at 50 °C. deMan, Ragosa and Sharpe (MRS) is the selective media used for culturing the *Lactobacillus* bacteria. The preparation of MRS agar was conducted by suspending 67 g of MRS medium in 1 L of distilled water. The medium was heated to boiling to dissolve the medium completely. The medium was sterilized by autoclaving at 121 °C for 15 minutes. The growing of colonies on the respective agar was further subculture on Nutrient Agar (NA) for cultivation. NA was prepared by suspending 28 g of NA medium with 1 L of distilled water and the mixture was boiled until the mixture dissolve completely. The mixture was sterilized by autoclaving at 121 °C for 15 minutes.

Carcass yield

After six weeks of rearing, six quails from each treatment were selected and killed by severing the jugular vein. The quails were scalded at 65 °C for 15 to 30 seconds. The carcass weight was measured after removal of feathers and blood prior to slaughtering. The carcass was eviscerated by cutting off the head and feet, and

removal of the internal organs (Chen et al., 2013). The carcass was then disjointed by making a forequarter cut and hindquarter cut. For the forequarters, a longitudinal cut was made starting from the first thoracic vertebra and extending posteriorly through the sixth thoracic vertebra cutting the keel into half. Meanwhile, the hindquarter was obtained by cutting from the seventh thoracic vertebra and extending posteriorly splitting the lumbar-sacral vertebra into half (Hudspeth et al., 1973). The weight of 20 individual carcass parts of each treatment was calculated and expressed as a mean weight.

Statistical analysis

One-way ANOVA model of SPSS 17.0 statistical program with Tukey's multiple comparison post-hoc test was applied to data analysis on carcass yield and microbial population. Probability values of less than 0.05 ($p < 0.05$) were considered significant. The data on carcass yield and microbial population were expressed as Mean ± Standard Error.

RESULTS AND DISCUSSION

Effect of probiotic, *B. subtilis (natto)* on microflora of feces and intestine in broiler quail

It has been suggested that when *Bacillus subtilis (natto)* is added in the poultry diet, it would associate with the gut wall and increase the population of natural beneficial bacteria such as *Lactobacillus*, which, at the same time may depressed the pathogenic microorganism such as *E. coli*. The maintenance of lactic acid bacteria such as *Lactobacillus* has been recognized to have beneficial effects on host's health and susceptibility to disease (Goldin 1998; Rizzardini et al., 2011; Patten & Laws 2015). Previous study by Hosoi et al. (2000) shows that exogenously added catalase as well as *B. subtilis (natto)* to cultures are effective in promoting the number of viable cells of two strains of lactobacilli. The current study showed that treatment 1, with 0.05% *B. subtilis (natto)* has significantly ($p<0.05$) difference with the control group towards the increasing amount of *Lactobacillus*. This indicate that small concentration of *B. subtilis (natto)* which is 0.05%, are enough to increase the number of beneficial bacteria, *Lactobacillus* in both feces and intestine of quail (see Table 1 and 2).

The present result showed negative or absence of *Salmonella* in all treatments including control (see Table 1 and 2). The negative or non-appearance of *Salmonella* in both intestine and feces would not justify if *B. subtilis* had considerable inhibitory action towards the infection and growth of *Salmonella* in quail's intestine and feces. But, Wang et al. (2006) reported *B. subtilis* takes large amount of oxygen while producing in intestinal tract, thus it can strongly inhibit the growth of aerobic pathogenic bacteria (*Salmonella*) and enhance the anaerobic bacteria such as *Lactobacillus*. Mashed diet used to feed the quails in the current study could be a reason that could inhibit the growth and development of *Salmonella.* Previous finding of Vandeplas et al. (2010) revealed that structure of the feed can help poultry to develop resistance to *Salmonella* infection. In addition, Huang et al. (2006) research on effects of feed form and feed particle sizes on *Salmonella* growth showed less cecal *Salmonella* in broiler chickens fed with mashed diets as compared to those fed with pelleted diets. Other factors such as regular sanitation, hygiene and good management practices could also be a reason lead to the absence of *Salmonella* in this study as described in the previous studies (Mead, 2004; Bjerrum et al., 2005).

The amount of *E. coli* in treatment 3 on feces was significantly ($p<0.05$) lowered than that of the control. Thus, the concentration of 0.35% *B. subtilis (natto)* are the most optimum concentration to lower the amount of *E. coli* in quail's feces. However, the amount of *E. coli* in quail's intestine does not affected by *B. subtilis (natto)* cultures (Table 2). *B. subtilis* was believed to eliminate *E. coli* in young chicken, in which, when given to day-old chicks 24 hours prior to challenge with *Escherichia coli* O78, resulted in significant reduction in colonization of the gastrointestinal tract and fecal shedding by the *E. coli* challenge (La Ragione et al., 2001). The suppression enhanced better growth of beneficial bacteria.

Table 1. Effect of probiotic, *B. subtilis (natto)* on microflora [log (CFU/ml)] of feces in broiler quail.

Bacteria Spp.	Treatments			
	Control 0.00%	Treatment 1 0.05%	Treatment 2 0.20%	Treatment 3 0.35%
E. coli	6.87 ± 0.25*	6.71 ± 0.25	6.63 ± 0.25	5.99 ± 0.25*
Lactobacillus	7.42 ± 0.18*	8.02 ± 0.18*	7.75 ± 0.18	7.61 ± 0.18
Salmonella	Negative	Negative	Negative	Negative

*In the same row, values with * mean significantly difference ($p<0.05$).

Table 2. Effect of probiotic, *B. subtilis (natto)* on microflora [log (CFU/ml)] of intestine in broiler quail

Bacteria spp.	Treatments			
	Control 0.00%	Treatment 1 0.05%	Treatment 2 0.20%	Treatment 3 0.35%
E. coli	7.04 ± 0.70	7.32 ± 0.70	6.10 ± 0.70	6.54 ± 0.70
Lactobacillus	5.88 ± 0.16*	6.37 ± 0.16*	5.99 ± 0.16	5.97 ± 0.16
Salmonella	Negative	Negative	Negative	Negative

*In the same row, values with * mean significantly difference (*$p<0.05$*)

The gastrointestinal (GI) tract are the most comprehensive exposed to variety potentially harmful substances. *E. coli* and certain related bacteria create about 0.1% of gut flora, and the major route is via fecal-oral transmission which can cause infection to the host. Thus, *E. coli* population in intestine could possibly increase due to several factors and mainly influenced by age, diet, gut location, rearing environment and interactions within bacteria communities (Wei et al., 2013; Yeoman & White, 2014). *B. subtilis* can maintain the balance of intestinal microenvironment and improved the feed conversion ratio by maintaining the intestinal beneficial bacteria and compete with pathogen for nutrients (Leser et al., 2008). Several studies showed that *B. subtilis* can secrete pathogen-suppressive substances that have bacteriostatic action on common pathogens such as *Staphylococcus aureus* and *E. coli* that were highly sensitive to sample concentrate and the bacteriostatic effect is equivalent to normal antibiotics (Abdelqader et al., 2013; Ushakova et al., 2013). Xiumei et al., (2004) and Stanley et al., (2014) reported that *B. subtilis* are capable to suppress *E. coli* while promoting anaerobic intestinal bacteria growth or live in symbiosis with them. Maruta et al., (1996) believed that after administration of *Bacillus subtilis* C-3102 causing reduction in incidence and level of *Campylobacter* and incidence of *Salmonella* in the intestinal tract of broiler.

Effect of probiotic, *B. subtilis (natto)* on carcass yield of broiler quail.

There was a significant ($p<0.05$) difference for the dressing percentage between the four groups. The dressing percentage (%) was 67.46 ± 3.64, 80.69 ± 3.64, 77.43 ± 3.64, and 81.77 ± 3.64 in control group, treatment 1, treatment 2, and treatment 3 respectively (see Table 3). There was significantly ($p<0.05$) increased in dressing percentage between control group, treatment 1, and treatment 3. The addition of probiotic, *B. subtilis (natto)* resulted in significantly ($p<0.05$) higher legs percentage, but has no significant ($p>0.05$) on the breast percentage. The breast percentage (%) was 36.95 ± 1.37, 36.19 ± 1.37, 38.00 ± 1.37, and 37.74 ± 1.37 in control group, treatment 1, treatment 2, and treatment 3 respectively. The legs percentage (%) was 31.21 ± 0.57, 33.39 ± 0.57, 34.14 ± 0.57, and 34.08 ± 0.57 in control group, treatment 1, treatment 2, and treatment 3 respectively. The results showed there was significant ($p<0.05$) difference in legs percentage between all groups.

Table 3 Effect of probiotic, *B. subtilis (natto)* on carcass yield of broiler quail.

Parameters	Treatments			
	Control 0.00%	Treatment 1 0.05%	Treatment 2 0.20%	Treatment 3 0.35%
Dressing percentage	67.46 ± 3.64*	80.69 ± 3.64*	77.43 ± 3.64	81.77 ± 3.64*
Breast percentage	36.95 ± 1.37	36.19 ± 1.37	38.00 ± 1.37	37.84 ± 1.37
Leg percentage	31.21 ± 0.57*	33.39 ± 0.57*	34.14 ± 0.57*	34.08 ± 0.57*

*In the same row, values with * mean significantly difference ($p<0.05$).

The quails in the groups having *B. subtilis (natto)* supplemented feed had a relatively significant difference in the carcass size when compared with the control group. Awad et al. (2009) found that no significant reduction in the carcass percentage when feeding broiler with probiotic supplemented diet while Chiang and Hsieh (1995) reported there is no significant difference were observed in breast yields among the groups fed with supplemented probiotic. However, a significant difference was observed in legs yields among the groups when fed with supplemented probiotic. Corrêa et al. (2000) and Santos et al. (2002) reported that broilers that received any kind of probiotic resulted in higher leg yield. However, no significant difference was observed in leg yield between control birds and those receiving additives (Henrique et al., 1998; Loddi et al., 2000). Kabir et al., (2004) reported that adding 2 g probiotic per each liter of water consumed by broiler chicken, would increase the efficiency in their thigh and breast as compare with the control group. Therefore, although adding probiotic in the diet would increase the carcass yield and differed positively in the leg yield, but the prime cut which is the breast were not different among the groups.

CONCLUSION

The probiotic in diet has increased the performance of the quail in terms of intestinal and fecal microflora by increasing the beneficial bacteria and suppressing the pathogenic bacteria. The use of probiotic in the diet could not be concluded to inhibit the infection of *Salmonella* towards the broiler quails, as other factors might play a vital role to hinder its growth and development. However, adding probiotic in feed shown a positive effect on the carcass yield in terms of dressing percentage and legs percentage.

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