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Influence of a Probiotic Containing *Lactobacillus fermentum* on the Laying Performance and Egg Quality of Japanese Quails

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Abstract: The objective of this study was to investigate the potential of *Lactobacillus fermentum*, isolated from the intestine of Japanese quails (*Coturnix japonica*), as a probiotic product. The influence of this probiotics preparation on the laying performance and egg quality of quails was evaluated in a 28-day experiment. A fully randomized design was used with four experimental treatments, namely standard feed, standard feed with antibiotic, standard feed with 5.27 x 10^8 cells/ml *L. fermentum* and standard feed with 2.35 x 10^9 cells/ml *L. fermentum*. The results showed that *L. fermentum* supplementation did not influence ($p>0.05$) egg quality parameters (haugh unit, % egg albumen, % egg yolk and egg shell thickness) and egg weight, but significantly improved ($p<0.05$) total egg production and lowered cholesterol content in egg yolk. In conclusion, *L. fermentum* may be used as a feed additive in Japanese quail diets to improve egg production and to lower egg cholesterol content.

Key words: Egg quality, performance, Japanese quail, probiotics, *Lactobacillus fermentum*

INTRODUCTION

Japanese quail (*Coturnix japonica*) eggs have a high potential to be developed as a cheaper source of protein, especially in developing countries. In addition to being cheaper and delicious, quail eggs are rich in protein and good sources of folate, vitamin B12, pantothenic acid, iron, phosphorus, riboflavin and selenium (Bing, 2011). However, quail egg is reported to be high in saturated fats and cholesterol (Anonymous, 2011). It is therefore necessary to explore ways by which egg cholesterol levels can be lowered.

In recent years, with growing family incomes, the demand for poultry products has increased in Indonesia. At the same time, consumers are also becoming increasingly concerned about the safety of poultry products. The major concerns are related to the presence of antibiotic residues in poultry products that can cause adverse effects on human health and the possible development of antibiotic resistant bacteria. One key strategy to replace the use of antibiotics in poultry diets is to feed microorganisms, which are known to exert beneficial effects in the gut, directly to the bird. Probiotics are live microorganisms which are supplemented to the feed in order to establish a beneficial gut microflora. Use of these microbes in diets for Japaneses quail may enhance the intestinal health by modifying the gut microflora. Probiotics not only deliver health benefits for the birds that consume them, but also for consumers who purchase the poultry products. In our laboratory, several strains of microbes have been successfully isolated from the intestinal tract of quails and characterized (Kalsum *et al*., 2008). From these microbial strains, the genus of *Bacillus* was selected for evaluation as a probiotic. This genus is known to produce several enzymes such as protease (Libertina *et al*., 2009) and amylase (Wardhani *et al*., 2009) and therefore it potentially can be exploited as a probiotic. The bacteria belonging to this genus had several dominant species of interest. Of these, *Lactobacillus fermentum* was identified as promising and considered for further evaluation as a probiotic (Kalsum *et al*., 2008). The spore-forming lactic acid bacteria belonging to this genus are stable, resistant to high temperatures and tolerant to bile salts.

Kalsum *et al*. (2010) reported that the supplementation of *L. fermentum* probiotic, isolated from quail intestine, improved the bacterial profile and health of the digestive tract of quail. This was due to the fact that *L. fermentum* can produce anti-bacterial compounds that could inhibit the growth of microbial pathogens such as *Salmonella typhimurium* and *Eschericia coli*. Such beneficial effects on gut flora may be expected to improve the production performance of quails.

Using lactic acid bacteria as probiotics is an alternative to the use of in-feed antibiotics as prophylactic and growth-promoting agents in poultry production. Previous studies on probiotics with laying hens have demonstrated positive responses with dietary supplementation (Li *et al*., 2006; Kalavathy *et al*., 2009), but no studies to date have investigated endogenous probiotics in diets for laying quails. The objective of the present study was to examine effects of the

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addition of a probiotics bacteria *L. fermentum*, isolated from quail intestine, on performance of laying quails, egg quality characteristics and egg yolk cholesterol content.

MATERIALS AND METHODS

The experimental protocol was approved by the Brawijaya University Animal ethics and welfare Committee.

L. fermentum was isolated from the intestine of Japanese quails and cultured in agar based on the formulations of deMan, Rogosa and Sharpe (MRS) for 48 hrs at 37°C. This medium supports luxuriant growth of lactobacilli from oral, fecal, dairy and other sources (Murray *et al.*, 1995). Then a series dilutions up to 10^{-7} in MRS liquid was performed, followed by spreading on MRS agar that containing 60 mg/kg Bromocresol Purple (BCP) as the pH indicator and incubated for 24 hrs at 37°C. Lactic Acid Bacteria (LAB) colony count was determined based on the change of media to be pale yellow at the location of the growth of bacterial colonies. The characteristic test confirmed that the isolates are LAB, gram positive, catalase negative and negative endospores. Isolation of the species was confirmed with the API 50 CH test Medium Kit (bioMerieux, 2001; Abegaz, 2006) and API 50 CHL medium. To determine it's feasibility as a probiotic, the candidate was then evaluated using several tests, including pH test, pathogen test and bile salt test.

A total of 160 laying quails, 150 days old, were randomly assigned to 16 cages (40 x 40 x 25 cm^3) of 10 birds each and then the cages were allocated to four treatment groups with four replications per treatment. The birds were housed in individual wooden battery cages, in a temperature controlled room at 73 °F. All birds had free access to feed and water. The photoperiod was 16 hrs of light per day throughout the experimental period according to Vatsalya and Kashmiri (2011). Prior to the introduction of experimental diets, all birds were fed *ad libitum* a standard diet (Table 1) for 10 days and egg production was monitored. During this period, it was found that the egg production was similar in the different groups and that the coefficient of variation did not differ ($p>0.05$).

The four experimental diets were as follows: standard feed, standard feed with 50 mg/kg zinc bacitracin, standard feed with 5.27 x 10^8 cells/ml *L. fermentum* and standard feed with 2.35 x 10^9 cells/ml *L. fermentum*. After confirming the cell counts in the laboratory using haemocytometer according to Henderson (2010), the probiotic was first mixed with corn and then added to the diet. Diets containing the probiotic were mixed at weekly intervals. The standard feed was formulated to meet or exceed the nutrient recommendations by National Standard Indonesia (SNI, 2006) and the diet composition is shown in Table 1.

Table 1: Composition (%, as fed) of the standard feed

Ingredient	
Yellow corn	52.48
Soybean meal	20.24
Fish meal	9.99
Rice bran	4.50
Limestone	4.89
Wheat pollard	4.40
Coconut cake	2.58
Vitamin-trace mineral premix[1]	0.50
L-lysine	0.26
Salt	0.12
DL methionine	0.04
Calculated analysis	
Metabolisable energy (kcal/kg)	2700.00
Crude protein (%)	20.00
Crude fat (%)	3.48
Crude fibre (%)	4.53
Calcium (%)	2.62
Phosphorus (%)	0.42

[1]Gram of premix contained: 2000 IU retinol, 400 IU cholecalciferol, 2 mg α-tocopherol, 1.2 mg riboflavin, 2 μg cobalamin, 200 μg pyridoxine, 100 μg vitamin K3, 1.5 mg calcium D-pantothenate, 5 mg choline chloride, 50 mg DL-Methionine, 3 mg nicotinic acid, 6.4 mg iron, 1.6 mg copper, 10 mg magnesium, 8 mg zinc, 6 mg manganese, 20 μg cobalt, 20 μg Iodine, antioxidant

Feed intake, egg production and egg weights were recorded daily during the experimental period that lasted 28 days. Egg mass was calculated by multiplied of egg production by egg weight according to Olgun *et al.* (2009). Feed Conversion Ratio (FCR) is a number indicating the amount of feed (gram) required by quail to produces one gram egg. FCR calculated by dividing the feed intake by egg mass. Egg quality parameters were measured on the Friday of each week. Egg weight, egg yolk and albumen weight percentages were determined in 10 eggs per replication according to the procedures of Christaki *et al.* (2011). Egg shell thickness was measured by micrometer according to the method of Balkan *et al.* (2006). Haugh unit was calculated from the logarithm of albumen height and transformed into a correction value of the function of egg weight based on Rodriguez *et al.* (2002). The height of albumen was measured by a spirometer according to Roberts (2004). Yolk extracts were collected from 10 eggs per replication and yolk cholesterol was analyzed according to the method of Subekti *et al.* (2006).

Data were statistically analyzed using the analysis of variance. Significant differences between treatment means were separated using Duncan's multiple range test according to Steel and Torrie (1992).

RESULTS AND DISCUSSION

The endogenous probiotic as feed additive that was used in this study was the lactic acid bacteria identified as *Lactobacillus fermentum*. The potential of this bacterial species as a probiotic was supported by

Table 2: The effect of *L. fermentum* administration on the performance of laying quails[1]

Treatment	Feed intake (g)	Egg production (%)	Egg mass (g)	Feed conversion ratio (g feed/g egg)
Standard feed	30.140	65.700[a]	7.6370	3.9460
Standard feed + 50 mg/kg zinc bacitracin	29.670	66.150[a]	7.7890	3.8350
Standard feed + 5.27 x 10^8 cells/ml *L. fermentum*	30.990	70.140[ab]	8.1520	3.8520
Standard feed + 2.35 x 10^9 cells/ml *L. fermentum*	32.050	75.520[b]	8.7570	3.6610
Pooled SEM	3.724	4.582	0.5856	0.5800

[a,b]Different superscripts in a column denote significant difference ($p<0.05$).

[1]Each value represents the mean of four replicates (10 birds/replicate)

Table 3: The effect of *L. fermentum* administration on egg quality parameters[1]

Treatment	Egg weight (g)	Egg yolk (%)	Egg albumen (%)	Egg shell (mm)	Haugh unit	Egg yolk cholesterol (mg/dL)
Standard feed	11.64	31.580	51.84	0.194	80.870	75.750[d]
Standard feed + 50 mg/kg zinc bacitracin	11.81	32.030	51.07	0.197	86.030	74.130[c]
Standard feed + 5.27 x 10^8 cells/ml *L. fermentum*	12.14	30.720	53.61	0.192	90.130	72.010[b]
Standard feed + 2.35 x 10^9 cells/ml *L. fermentum*	11.59	31.950	51.68	0.192	89.510	71.030[a]
Pooled SEM	0.84	2.087	3.38	0.017	5.009	0.127

[a,b,c,d]Different superscripts in a column denote significant difference ($p<0.01$).

[1]Each value represents the mean of 160 observations

Jans (2007). Most of the probiotics used as feed additive in broiler diets belong to *Lactobacillus*, bacteria and *Bifidobacterium*.

The effect of *Lactobacillus fermentum* supplementation performance parameters of Japanese quails is summarized in Table 2. Statistical analysis showed that *L. fermentum* supplementation had no effect on feed intake and egg mass ($p>0.05$). Although feed intake and egg mass observed in birds fed diets containing high level of L. *fermentum* diet treatments were numerically higher than those fed the standard feed or zinc bacitracin treatment, the differences were not significant ($p>0.05$). Similar findings have been reported by Cakir *et al.* (2006) who reported no effect of dietary supplementations of probiotic-prebiotic, organic acid and avilamycin on the growth performance, feed intake, feed efficiency and proportional organ weights of growing Japanese quails. In contrast, Zeweil *et al.* (2006) found that the supplementation of 1.0 or 2.0 g/kg probiotic (Lacto-Sac) significantly improved on egg production, egg mass, egg weight and feed conversion ratio as compared to control laying Japanese quail hens.

Probiotic supplementation did not affect ($p>0.05$) feed conversion ratio, but significantly improved egg production by 14.95%. Egg production was highest in birds fed the standard feed containing the high dose of probiotics (2.35 x 10^9 cells/ml) and this was significantly higher ($p<0.05$) than those receiving the zinc bacitracin diet. This observation highlights the potential of *L. fermentum* to improve egg production and this may be due to its role in improving the apparent metabolizable energy and digestibility of protein (Wu *et al.*, 2004; Centeno *et al.*, 2007).

The data presented in Table 3 show that the addition of *L. fermentum* to quail diets did not significantly ($p>0.05$) affect the quality of quail eggs, except for cholesterol levels which were lower ($p<0.01$) in the probiotic diets. These findings are consistent with those of Guclu (2011) that the use of probiotics did not have a significant impact on egg weight, albumen index, yolk index and haugh unit.

The use of *L. fermentum* lowered the cholesterol content of quail eggs. Similarly, Haddadin *et al.* (1996) reported that the inclusion of *Lactobacillus acidophilus* in laying hen diets at levels up to four million viable cells per gram feed of decreased cholesterol values in egg yolk by 18.8%. Mahdavi *et al.* (2005) also reported that the use of *Bacillus subtilis* and *Bacillus licheniformis* decreased egg cholesterol of laying hens. Kankaanpaa *et al.* (2004) speculated that probiotics can increase the secretion of enzymes in the digestive tract that can destroy a specific substrate or anti-nutrient in the feed. Another mechanism is by inhibiting reactions that produce carcinogens, stimulating the increase of detoxification reactions and synthesizing the essential ingredients. Abdulrahim *et al.* (1996) observed that *Lactobacillus acidophilus* improved egg production, feed conversion and reduced the cholesterol content in eggs, whereas zinc bacitracin had no effect when administered alone. In combination with the probiotic, zinc bacitracin had an adverse effect on otherwise beneficial activity of the culture. Nahashon *et al.* (1994) also found that *Lactobacillus acidophilus* has the ability to break down simple carbohydrates into lactic acid. As lactic acid is increased, pH of the gut environment is decreased and the growth of pathogenic microorganisms is restricted. Sulthan and Abdul-Rahman (2011) reported that the probiotic treatment significantly decreased serum triglyceride levels and increased serum uric acid levels.

Conclusion: Probiotics have great potential to beneficially affect the gut microflora and hence improve gut health in poultry. The present data confirms the potential of *L. fermentum*, isolated from the intestine of Japanese quails, as a probiotic. The administration of *L. fermentum* in laying quail diets significantly improved egg production and lowered the cholesterol content of egg yolk.

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