Effect of Probiotic Containing *Enterobacter* Spp., *Bacillus* Spp., *Cellulomonas* Spp., *Actinomyces* Spp. Supplementation on The Laying Performance and Egg Cholesterol of Quail

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Abstract. The purpose of this study was to know the effect of probiotic supplementation to feed consumption, egg production, egg weight, feed conversion, feed efficiency and egg cholesterol. 80 quails of *Coturnix coturnix japonica* at 14 weeks of age were completely randomized into five treatments, each treatment consisted of four replication and each replication consisted by five heads. T₀ as a control without probiotic supplementation, T₁ with the addition of 1 ml probiotic/kg of feed, T₂ with the addition of 2 ml probiotic/kg of feed, T₃ with the addition 1 ml probiotic/liter drinking water and T₄ with the addition of 2 ml probiotic/liter drinking water. The results showed that the probiotic supplementation gave a significant effect (p<0.05) to feed consumption, feed conversion, feed efficiency and egg cholesterol, but couldn’t effect (p>0.05) egg weight and Quail Day Production. T₄ showed the highest significantly different in feed conversion, feed efficiency and egg cholesterol. It could be better to give 2 ml probiotic containing *Enterobacteria* spp., *Bacillus* spp., *Cellulomonas* spp., and *Actinomyces* spp. in drinking water for the best result.

**Keywords:** Probiotic, production performance, egg cholesterol, quail

1. Introduction

Japanese quail, the smallest avian species is getting more importance for commercial egg and meat production with high rate of egg production. It has marked advantages such as fast growth, early sexual maturity, high rate of egg production and short generation interval [1].
Livestock feed nowadays become very expensive resulting in decrease in livestock production [2]. Recently the baned on the use of antibiotic as a growth promoters (AGP) in animal feeds, due to their residual effect in animal products as well as the development of bacterial resistance in animals and human body, have make a way to find alternatives of antibiotics [3]. It is generally accepted that direct-fed microbials (DFMs), defined as a source of viable microorganisms, as an alternative to antibiotics [4]. Probiotics or direct-fed microbials (DFM) are live microorganisms, when administered in adequate amounts, give a health benefit on the host [5]. Most probiotic strains which used in livestock are members of the *Bacillus* and *Enterococcus* genera [6].

Feeding Probiotic can reduce feed conversion [7]. In addition to increase the quantity of eggs produced, the fact that this probiotic can reduce egg cholesterol level may encourage people especially in developing countries to consume more eggs and enjoy good health as well as other benefits derived from egg [8].

The probiotic use in quail through feed and water, then expect that the consumption of probiotics can improve the efficiency of feed, then increase egg production. The purpose of this study was to know the effect of probiotic containing *Enterobacteria*, *Bacillus* spp., *Cellulomonas* spp., and *Actinomyces* spp. supplementation to feed consumption, egg production, egg weight, feed conversion, feed efficiency and egg cholesterol.

2. Materials

The isolates of *Enterobacteria* spp., *Bacillus* spp., *Cellulomonas* spp., and *Actinomyces* spp. isolated from bovine rumen fluid waste of Surabaya Abbatoir, Indonesia, from previous study [9]. Organic quail feed was made from organic grain and other crop without any antibiotic, chemical composition and other prohibited materials. A total of 80 laying quails (14 weeks) from Bojonegoro, Indonesia.

3. Methodology

3.1 Study Area and Farm Management

The research was conducted in the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Probiotics used in this study contained cellulolytic and proteolytic bacteria consisted of *Enterobacteria*, *Bacillus* spp., *Cellulomonas* spp., and *Actinomyces* spp.

A total of 80 laying quails (14 weeks) were randomly assigned to five treatments, with four replication each treatment and five heads each replication, the treatments were as follow: T0 was control with 100% quail feed, T1 was quail feed with 1 ml probiotic/gram of feed, T2 was quail feed with 2 ml probiotic/gram of feed, T3 was quail feed with 1 ml probiotic/liter water drinking and T4 was quail feed with 2 ml probiotic/liter water drinking

The probiotic liquid, dissolved in water (free chlorine and other antiseptics), then allowed to stand for 24 hours without aeration. The probiotic solution (T1 and T2) sprayed evenly into 100 kg of feed and then left the feed dried and the other probiotic solution (T3 and T4) then stirred evenly into drinking water.

3.2 Data Collection and Statistical Analysis

Feed intake, egg production, egg weight were recorded daily during the experimental period that lasted 28 days. feed intake was a number of feed given divided with feed which not consumed. Egg weight was calculated by egg weight by egg production. Egg production was calculated in quail-day production (QDP) with the ratio of eggs produced per day and the total of females quail in population in 100%. Feed conversion was calculated as a ratio of feed intake by the egg weight. Feed efficiency was calculated as a ratio of egg weight by feed intake. The egg cholesterol measured after four week of treatment, with 5 randomized eggs each treatment, with enzymatic colorimetric method.
3.3 Statistical Analysis
Data analysis was performed by Analysis of Variance (ANOVA). If the result showed differences or significantly different then continued with Duncan Multiple Range Test [10]. Statistical analysis performed with SPSS for Windows 21.0.

4. Results and Discussion
The results showed that the probiotic supplementation gave a significant effect (p<0.05) to feed consumption, feed conversion and feed efficiency, where T4 was significantly the highest (p<0.05). Balanced gastrointestinal microflora were major functional effects attributed to the consumption of probiotics [11], then the utilization of selected probiotics might improve the metabolism of the host animals in various ways, there were absorptive capacity, protein metabolism and energy metabolism [12], then improved the digestion of food and minimized feed consumption [13]. This result in line with [14] reported that addition of probiotic to broiler chicken diets decreased feed conversion significantly. [15] reported that the supplementation of probiotics to laying hens improved hen performance, there were feed efficiency, egg production and egg quality. [16] reported that diets with probiotic supplemented showed a potential to improve feed efficiency. Moreover, the supplementation via drinking water might also contribute to the improvement, which consistent to previously studies that probiotic administration via drinking water appeared to be superior than in-feed supplementation method [17]. The result showed in Table 1.

Table 1. The result of feed consumption, feed conversion, feed efficiency in 4 week of treatment

| Treatment | Feed consumption (g) ± SD | Feed conversion ± SD | Feed efficiency ± SD |
|-----------|---------------------------|----------------------|----------------------|
| T0        | 23.0227 ± 0.01673         | 2.1239 ± 0.01321     | 47.5178 ± 0.00837    |
| T1        | 21.4060 ± 0.02074         | 1.9644 ± 0.01304     | 48.6541 ± 0.00932    |
| T2        | 22.0545 ± 0.01581         | 2.0593 ± 0.01140     | 50.5962 ± 0.01893    |
| T3        | 22.4333 ± 0.01791         | 2.0579 ± 0.01472     | 48.3588 ± 0.01734    |
| T4        | 21.3587 ± 0.01304         | 1.9645 ± 0.02550     | 50.3900 ± 0.0342     |

a, b, c, d: different superscript in the same column showed a different effect (p<0.05)

The results showed that the probiotic supplementation couldn’t effect (p>0.05) Quail egg weight and Quail Day Production, where the treatment showed lower egg weight and egg production. Egg production was costly in terms of energy and protein. The required energy for egg formation may be derived from daily feed intake. Daily feed intake was more important source of nutrient for small birds like quail than body reserved. If energy or protein was limited, birds could compensate by reduced egg size or the number of eggs laid [18]. The effectiveness of probiotic application might depend on factors such as microbial species composition (example single or multistrain), liveability, supplemental administration dose, method and frequency of application and diet composition [16]. Although egg weight was a highly heritable trait, the beneficial effect of probiotics on egg weight may be attributable to a favorable environment in the intestinal tract, which might help to assimilate more nutrients [15].

This result contrast with [19] reported that egg production improved by approximately 50% on supplementation with 10⁷ cfu/g of probiotic under normal environmental conditions. [20] reported that there were significantly improvements in egg production and egg quality when 500 mg of B. subtilis culture/kg was added to the diets. [21] observed significant (P < 0.05) improvements in feed efficiency and egg production when 20-week-old layer hens were fed a probiotic mixture that included Lactobacillus strains. Similarly, [22] reported that probiotic (BioPlus 2b) fed to 27-week-old Brown-Nick layers could increase egg production. [23] reported that Lactobacillus salivarius + Bacillus subtilis, showed significantly increased egg production and daily egg yield as well as decreased feed conversion. [24] reported that the inclusion of 0-6% liquid metabolite combinations, produced from three L. plantarum strains, demonstrated the best effect in improving the hens’ egg production. [25] reported that the inclusion of Bacillus licheniformis in the hen diet was
effective to increase egg production. [26] reported that supplementation of feed mixture with probiotics increased egg production. [23] reported that dietary supplementation of 0.01% probiotic improved egg production and egg quality. The result showed in Table 2.

Tabel 2. The result of egg weight and QDP in 4 week of treatment

| Treatment | Egg weight (g) ± SD | Quail day production (%) ± SD |
|-----------|--------------------|-----------------------------|
| T0        | 10.9072 ± 0.01140  | 55.7677 ± 0.02074           |
| T1        | 10.8276 ± 0.15210  | 52.7273 ± 0.01581           |
| T2        | 10.7244 ± 0.02074  | 41.8222 ± 0.01140           |
| T3        | 10.8485 ± 0.02341  | 55.0300 ± 0.01801           |
| T4        | 10.7567 ± 0.01346  | 50.0300 ± 0.01721           |

The results showed that the probiotic supplementation gave a significant effect (p<0.05) to egg cholesterol. Probiotics were reported to have hypocholesterolemic [27]. Some lactic acid bacteria might secrete high-activity bile salt hydrolase (BSH) during metabolism, and this secretion could considerably affect cholesterol levels [28].

Probiotic supplementation might also played a good role to change the lipid metabolism as various studies showed that probiotics could reduce total cholesterol and triglyceride contents of egg yolk [29]. [30] reported that Probiotics reduced cholesterol concentrations in egg yolk. [22] reported that supplementation probiotic Bacillus licheniformis and Bacillus subtilis decreased egg yolk cholesterol. [19] reported that the cholesterol content of eggs produced by probiotic (Lactobacillus culture) fed hens was significantly lower by 15.3% and 10.4% when compared to those of the control hens at 24 and 28 weeks of age, respectively. Other researchers [16] observed also beneficial effects of the tested probiotic on egg quality such as a lower cholesterol content. The result showed in Table 3.

Tabel 3. The result of egg cholesterol in 4 week of treatment

| Treatment | Egg cholesterol (mg/g) ± SD |
|-----------|-----------------------------|
| T0        | 19.0222 ± 0.01064           |
| T1        | 6.7600 ± 0.01876            |
| T2        | 6.9302 ± 0.02134            |
| T3        | 13.5697 ± 0.01924           |
| T4        | 12.4480 ± 0.01856           |

5. Conclusion
It can be conclude that the probiotic can’t increase quail production and egg weight, but can decrease the feed consumption, feed conversion, egg cholesterol, then increase feed efficiency. The administration of 2 ml probiotic/liter drinking water show the best result.

Authors’ Contributions
WPL designed the research. NH and SH helped in designing the research. ARD and AF carried out the data collection and assisted in manuscript preparation; WPL, BK and ADA collected materials for manuscript. All authors have read and approved the final manuscript.

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