

Original Article

Egg quality and production by the addition of dried consortium probiotics on late-phase laying hens

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Abstract

Objective: Lactic acid bacteria are known to improve performance and gut health of laying hens. Several potential microbiotas that could enhance gut health are *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium sp.* Probiotics are microorganisms that play a role in improving the ecosystem of intestinal flora, which affects nutrient absorption process along with metabolism, and increase the production of eggs. This study aimed to determine the effect of the microbiota consortium in probiotic powder on egg quality and production for late-phase laying hens.

Methods: This research was done on January-April 2022 and used 40 Lohmann brown laying hens aged 70 weeks. The treatments given were 0, 2, 3, and 4% probiotic powder in laying hens' feed. Egg samples were collected every week, and parameters observed in the study were egg quality consisting egg weight, egg volume, and egg weight composition such as yolk weight, egg white weight, eggshell weight, yolk index, haugh unit, and egg production.

Results: The result of the study showed that the addition of probiotic powder could not enhance the egg quality ($P>0.05$) based on egg weight, volume, yolk index, haugh unit, and egg weight composition. However, a 4% level of probiotic powder could improve egg production significantly ($P<0.01$).

Conclusions: Hence, we can conclude that probiotic powder could be used as a feed additive to improve egg production in late-phase laying hens.

Keywords: Egg production; Egg quality; Laying hens; Probiotic powder

INTRODUCTION

Eggs have been one of the biggest contributors to fulfilling protein needs in Indonesia due to their rich nutritional value at an affordable price. Eggs contain complete nutrition to fulfill human needs such as protein, carbohydrates, lipids, vitamin, and minerals [1]. Therefore, Indonesia has a large number of layer farms and needed strategies to improve both production and quality of the eggs in laying hens.

Traditional laying hens in Indonesia are culled if their egg production is less than 50%, this condition is often found in late-phase laying hens aged more than 70 weeks [2]. Along with

the increased age, several health conditions were also decreased and caused the poor quality of eggs and production. Although the decline in egg production and quality, traditional layer farms did various efforts to improve their productivity in the late laying period to increase the efficiency of laying hens before rejected, one of them is by giving natural feed additives such as probiotic powder.

Probiotics is one or a consortium of micro biota that gives beneficial effects to the hosts. Probiotics is known to improve digestibility and gut health, increase immunity, reduce infection by pathogenic bacteria, increase enzyme activity and improve intestinal morphology [3,4]. The

beneficial function of probiotics will be more efficient if given in powder form to improve feed efficiency, easier to digested and absorbed by the gastrointestinal tract [5].

Lactic acid bacteria has the ability to secrete antibacterial substances such as lactic acid, bacteriocin, and active peptides that could inhibit the growth of pathogenic bacteria, and metabolite substances with antioxidant activities such as butyrate and folate [6,7]. Antioxidants produced by the probiotics are useful to reduce free radicals that causes oxidative stress [8]. Oxidative stress is known to decrease the number of follicles and affects egg production, egg quality traits, yolk lipids, and cholesterol contents. There have been many reports that antioxidants provide a beneficial effect on egg production [9]. Yogurt can be used as a probiotic because it contains several lactic acid bacteria such as *Streptococcus*, *Bifidobacterium*, and *Lactobacillus* [10].

Several previous studies related to giving probiotics to laying hens had a positive effect on their production. A previous study by [11] showed that the consortium of probiotic powder consisting of *Lactobacillus*, *Bifidobacteria*, and *Streptococcus thermophilus* was able to expand the surface and height of the intestinal villi and enhance the nutrient absorption process along with metabolism. It would increase the production of eggs. [5,12] reported that *Lactobacillus acidophilus* could increase protein digestibility, protein plays an important role in binding calcium in the form of calcium binding protein (CaBP), this complex is able to facilitate calcium absorption and store it to form egg shells and improve the quality of the shell structure. Besides the calcium absorption, [13] also explained that protein content correlates with egg weight and the protein as well as energy are required for the growth and production of laying hens.

Therefore, further research is needed to find out the effect of four combination microbiota from yogurt on egg quality and production of laying hens, specifically in their late phase.

MATERIALS AND METHODS

Experimental site

This research was done on January – April 2022. Production process and viability test were done on January – February 2022 in the Central Laboratory of Padjadjaran University, and the

feeding trial was done on March – April 2022 in an open house laying hens farm with a battery cage system in Sukarapi Village, Sumedang District, West Java. Egg samples were collected every week and analyzed immediately in the Laboratory of Poultry Production, Padjadjaran University.

Production of probiotics

Probiotics used were based on yogurt containing consortium microbiota consists of 5% (v/v) *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium sp.* Consortium microbiotas were inoculated in De Man Rogosa and Sharpe (MRS) medium and then incubated at 45°C for 14 hours. After the incubation process, probiotics were added to a pasteurized cow milk then homogenized. Fermentation process were done for 14 hours at the room temperature. The liquid fermented milk added maltodextrin, skimmed milk, and sterile distilled water as an encapsulation material with a comparison of 1:2 of the total volume of the solution. Maltodextrin worked as a coating material to reduce the damage that caused by external factors such as extreme temperature changes like spray drying [14]. Then the probiotics were dried using the spray drying method. Spray drying process were done with an inlet temperature of 160°C and outlet temperature at 65-70°C to produce fermented milk in powder form. Viability test were done after the spray drying process. The result of the viability test is shown in Table 1.

The data (Table 1) showed that viability in the microbiota of the probiotic powder is suitable with the standard number of lactic acid bacteria in yogurt based on [15], 1.6×10^7 CFU/g.

Feeding trial

This study used 40 laying hens of Lohmann brown strain aged 70 weeks. Five treatments were tested in each group. Probiotics are added to feed. The treatments include P0 (basal ration), P1 (basal ration with 2% of probiotic powder/kg of feed), P2 (basal ration with 3% of probiotic powder /kg of feed), and P3 (basal ration with 4% of probiotic powder/kg of feed).

Basal ration consists of corn, rice bran, concentrate, and mineral mix. Nutrient content and gross energy in the basal ration and probiotic

Table 1. Viability test on probiotic powder

		Number of Dilutions										SPC
10 ⁻²	Ave rage	10 ⁻³	Ave rage	10 ⁻⁴	Ave rage	10 ⁻⁵	Ave rage	10 ⁻⁶	Ave rage	10 ⁻⁷	Ave rage	Score (CFU/g)
>300	>300	>300	>300	344	316	164	160	11	148	9	6	1.6 x 10 ⁷
>300	>300	>300	>300	288		156		284		3		

Source: Central Laboratory of Padjadjaran University (2022).

powder are shown in Table 2. Probiotics are mixed in the ration and given to laying hens for six weeks.

Data collection and egg quality analysis

Egg data were collected from 40 late-phase laying hens every week. The data collection was conducted for 4 weeks. Parameters observed in the study were egg weight, egg volume, and egg weight composition such as yolk weight, egg white weight, eggshell weight, yolk index, haugh unit, and egg production. Egg weight was obtained by weighing eggs from each treatment with a digital scale. Egg volume was measured by using Archimedes principle [16], by putting the egg into a glass of water and measure the volume of the spilled water. Egg weight composition was determined by breaking an egg and weighing each composition (yolk, egg white, and eggshell) with a digital scale. The yolk index was calculated by dividing the yolk height and the diameter of the egg and multiplying it by 100 [17]. Haugh unit was calculated using a formula $HU = 100 \times \log(AH - 1.7 \times EW^{0.37} + 7.57)$ [18]. Egg production was recorded daily.

Statistical analysis

The study used an experimental method with Completely Randomized Design (CRD) using four treatments (0, 2, 3, and 4% probiotics) and each treatment has five replications. Statistical

analysis of the data used Analysis of Variance. The significant differences between different treatment means were evaluated using Duncan’s test by considering differences significant at $P < 0.05$.

RESULTS

The addition of probiotic powder in feed did not have a significant effect ($P > 0.05$) on egg quality. Based on the results (Table 3), showed that probiotic powder did not have a significant effect on egg weight ($P > 0.05$), but there was an increase in P1 treatment. Similar results were shown on egg volume (Table 3). The addition of all treatments of probiotic powder did not have a significant effect ($P > 0.05$) on egg volume, but there was an increase in P1 compared to the control treatment.

Administration of probiotic powder did not have a significant effect ($P > 0.05$) on the yolk index or haugh unit. In the data shown (Table 3), there was an increase in the yolk index treated with P3 when compared to the control treatment, while in haugh unit they didn’t show any increase, but it was still within the standard value. The addition of probiotic powder was also unable to improve egg composition such as eggshell weight, yolk weight and egg white ($P > 0.05$) as shown in Table 4, but there was an increase in eggshell and yolk weight with the probiotic powder addition treatment when compared to the control treatment. The results of the P3 treatment provided

Table 2. Nutrient content and gross energy of probiotic powder and the ration of each treatment

No.	Sample	Water	Ash	Crude Protein	Crude Fiber	Crude Fat	Carbohyd rate	Gross Energy (Kkal/kg)
1	Probiotic powder	9.77	5.58	27.50	3.44	13.66	49.82	4,051
2	P0	11.20	14.43	17.06	5.58	10.53	52.40	3,867
3	P1	10.83	15.80	17.00	5.76	8.44	53.00	3,733
4	P2	10.83	15.45	17.82	6.17	8.08	52.48	3,743
5	P3	10.45	15.31	17.47	6.40	9.13	51.69	3,811

Source: Laboratory of Ruminant Animal Nutrition and Animal Feed Chemistry, Faculty of Animal Husbandry, Padjadjaran University (2022).

Table 3. Effect of the addition of probiotic powder on egg weight, egg volume, yolk index and haugh unit in late-phase laying hens

Treatments	Egg Weight (g)	Egg Volume (mL)	Yolk Index	Haugh Unit
P0	71.28±1.92	57.05±1.78	0.392±0.04	85.79±5.48
P1	71.49±5.18	57.25±4.26	0.389±0.04	74.92±5.56
P2	67.17±1.59	52.53±3.56	0.392±0.04	83.66±7.92
P3	65.82±2.71	51.65±3.31	0.398±0.01	83.93±11.09

Source: Laboratory of Poultry Production, Faculty of Animal Husbandry, Padjadjaran University (2022).

Table 4. Effect of the addition of probiotic powder on eggshell, yolk and egg white weight in late-phase laying hens

Treatments	Eggshell Weight (g)	Yolk Weight (g)	Egg White Weight (g)
P0	7.17±0.30	16.82±0.37	48.62±3.72
P1	7.04±0.52	17.05±0.98	48.53±3.86
P2	6.90±0.28	16.26±0.68	44.90±1.47
P3	7.20±0.51	16.26±0.49	42.95±1.69

Source: Laboratory of Poultry Production, Faculty of Animal Husbandry, Padjadjaran University (2022).

an increase in eggshell weight, while the P1 treatment was the highest in yolk weight.

The results of probiotic powder administration on egg production are shown in Table 5. The administration of probiotic powder in laying hens had a significant effect ($P < 0.01$) on egg production. The addition of probiotic powder could increase the number of egg production, and highest results were seen in the P3 treatment. P3 treatment can increase egg production by 25% (63.69%) compared to the control (38.69%). This result indicates that the administration of 4% probiotic powder could effectively increase egg production.

Table 5. Effect of the addition of probiotic powder on egg production in late-phase laying hens

Treatments	Egg Production (hen-day %)
P0	38.69±8.87 ^a
P1	54.76±9.80 ^{ab}
P2	52.38±7.59 ^{ab}
P3	63.69±14.00 ^b

Source: Laboratory of Poultry Production, Faculty of Animal Husbandry, Padjadjaran University (2022).

Note: Superscript showed a high significant difference ($P < 0.01$)

DISCUSSION

Probiotics worked to decrease number of pathogenic bacteria in the digestive tract, so the nutrients would be optimally used for egg quality. However, age of laying hens also affects

the quality of the eggs because as the age increases, the egg weight will slightly increase but not followed by the quality [19]. A prominent sign of aging is represented by several chronic inflammatory and ovarian aging, and ovarian aging is considered to be one of the causes that contributes the decline of egg production [20]. The decline of producing eggs in late-phase laying hens is shown from their inconsistent production and quality. According to [19], age, laying period, and health status could determine the production and egg quality in layers. Therefore, although previous studies showed that probiotics might increase nutrient absorption and thus improve egg production and quality, the effect may vary and needs a specific mechanism for further investigation.

Similar to the egg weight result, the administration of probiotics did not affect the egg volume. This result may be caused by the linkage between egg volume and egg weight. [21] explained that egg volume affected by the shape and weight of the eggs, and this is also influenced by several factors, such as the nutrition of the feed, the environment, and the health condition of laying hens. While the health of the oviducts has gradually deteriorated in late-phase laying hens. The condition of the oviduct in egg production also affects whether the shape of the egg will be elliptical or asymmetrical, and the egg shape tends to affect volume [22].

The administration of probiotics also had no significant effect on the egg yolk index and haugh unit. No significant effect on egg yolk index

and haugh unit may be caused by the various effects of probiotics in late-phase laying hens, specifically in protein absorption. The egg yolk index and haugh unit are affected by protein content in the feed. The amino acids play a role in balancing the contents of ovomucin and lecithin which affect the condition of egg white in eggs, thus affecting haugh unit value [23]. However, while based on a previous study by [23] showed that probiotics could enhance protein absorption that may affect haugh unit and yolk index value, probiotics in late-phase laying hens may also act to lessen destructive effects on intestinal health and functions, since intestine health issues were thought to be a major contributing factor to poor laying performance in late-phase hens, according to [24]. These include maintaining the value of the Haugh unit and a yolk index within the standard range. Therefore, although there is no significant effect, both yolk index and haugh unit value are still in a normal range. The standard value of fresh egg yolk index according to [25] ranges between 0.33-0.52, while the haugh unit number range between 74-79, and the higher the values are known as having a better quality [26].

The composition of the egg consists of eggshell weight, egg yolk, and egg white weight measured to determine the internal quality of the eggs. The administration of probiotic powder did not have a significant effect on the weight of the egg composition. The positive effects of probiotic powder may not play an effective role because the nutrients can be used for other needs besides maintaining its quality. According to [27], conditions of laying hens such as age, hormonal status, and stress levels could affect egg composition.

Despite of no significant effect on egg quality, the addition of probiotic powder gave a significant effect on egg production. [28] explained that the final product represents how effective the probiotic works, with the intention to improve environmental conditions in the digestive tract, and to increase enzyme activity and the immune system. Its effectiveness can be seen by the increase in performance when compared to the control treatment. Probiotics also have an impact on the endocrine system, which improves hormone function, specifically FSH and LH [29]. FSH hormone plays a role in increasing the size of the follicle, and the FH

hormone works to increase ovulation and increase egg production [30].

CONCLUSION

In conclusion, the administration of probiotic powder had no significant effect on egg quality based on egg weight, egg volume, and egg weight composition, such as yolk weight, egg white weight, eggshell weight, yolk index, and haugh unit. However, the addition of 4% probiotics on late-phase laying hens is the best result and could improve 25% of egg production compared to the control.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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