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Research Article
Effect of Lactic Acid and pH of Probiotic Yogurt on Peak Production of Laying Hens

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Abstract

Background and Objective: Probiotic yogurt is beneficial for laying hens because it can improve the animal's hematological status which will improve livestock health, therefore it is hoped that probiotic yogurt can increase the production of laying hens. This research was conducted to determine the lactic acid levels and pH of probiotic yogurt, probiotic yogurt's effect on feed conversion ratio and total production of laying hens. **Materials and Methods:** The research was carried out using experimental methods using a Completely Randomized Design (CRD) with 5 treatments and 8 replications so the total sample was 40. The treatment consisted of P0: Basal ration; P1: Basal diet+2% probiotic powder B1 (*Bifidobacterium* spp. and *L. acidophilus*), P2: Basal ration+3% probiotic powder B1, P3: Basal diet+2% probiotic powder B2 (*L. bulgaricus*, *S. thermophilus*, *L. acidophilus* and *B. bifidum*) and P4: Basal ration+3% probiotic powder B2. The data were analyzed using Analysis of Variance (ANOVA) and followed by Duncan's Multiple Range Test. **Results:** Lactic acid content in probiotic yogurts B1 is 0.945% and B2 is 0.638%. Based on the results of statistical analysis using the variance test, show that giving probiotic powder to laying hens has a significant effect on the feed conversion ratio and has no significant effect on the production of laying hens. **Conclusion:** Based on the results of statistical analysis using the variance test, it shows that giving probiotic powder to laying hens has no significant effect on the production of laying hens during the peak period.

Key words: Laying hens, probiotic powder, lactic acid, pH, egg production, feed conversion ratio

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Laying hens are a type of poultry that are kept specifically for their ability to produce eggs. Laying hens have been bred for increased performance, reduce stress level and improve gut health, impacting the production with good environmental conditions[1]. Eggs are a livestock commodity that is very popular as a source of animal protein. Chickens are susceptible to disease, such as stress when heat stress is contaminated with microorganisms (bacteria, viruses and parasites) which disrupt the immune system and body metabolism. Infection of laying hens with *E. coli* and other pathogen bacteria can result in a significant decrease in egg production[2]. In chicken farming, feed is the highest component of production costs, around 70-80% of the total production, so feed must be used efficiently. Laying farms require innovation to produce quality and efficient livestock products. Probiotics given in the layer I phase to laying hens (31-week-old chicken) can help maintain chicken intestinal health and increase egg production. Giving probiotics to laying hens can help improve egg production in laying hens[3].

Probiotics can repair intestinal villi to increase absorption, besides that they can also increase metabolism and hormone synthesis which will increase the production of laying hens. Yogurt is a probiotic that contains lactic acid, bacteriocins and antioxidants which can increase digestibility and production in laying hens by suppressing the growth of pathogenic bacteria. Probiotics are live microbes that, when given in sufficient quantities, will provide health benefits to livestock that consume them[4]. The lactic acid content in yogurt can inhibit the growth of pathogenic bacteria because of the presence of non-pathogenic bacteria and the pH decreases so that pathogenic bacteria cannot survive.

In previous research, the use of bacteria is *Lactobacillus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*[5]. This research also uses these bacteria as cultures. The higher the number of microorganisms used, the faster the probiotic yogurt fermentation will be because more substrate is used. After the drying process, the total lactic acid bacteria was 1.6×10^7 CFU g^{-1}. These bacteria can produce lactic acid, bacteriocins and enzymes which can increase the digestibility and production of laying hens. The bacterial consortium utilizes lactic acid which is beneficial for the livestock's digestive tract. Lactic acid produced by lactic acid bacteria (LAB) will lower the pH thereby inhibiting pathogenic microbial contamination. Probiotics change the abundance and activity of microbes, which ultimately regulate the balance of the flora ecosystem in the small intestine. This affects the host's metabolism and health[6]. In addition, probiotics promote the production of digestive enzymes and antibacterial substances that prevent the development of harmful bacteria[7]. Several researchers have administered probiotics with *Bacillus* spp., *Lactobacillus* spp. and *Bifidobacterium* spp., strains to late-phase laying hens. So, if chicken digestion is healthy then absorption will be better and cause production to increase. Therefore, it is necessary to research the lactic acid content and pH of probiotic yogurt and their effect on feed conversion and the total production of laying hens.

MATERIALS AND METHODS

Study area: This research was conducted in June to August, 2023 at the Biochemistry and Food Chemistry Laboratory of the Department of Chemistry and Faculty of Animal Husbandry, Universitas Padjadjaran.

Probiotic yogurt preparation: The bacteria used were obtained from Nanobio Laboratory. There are two consortiums used in this study, namely B1 and B2. The probiotics that will be used for the B1 consortium are *Bifidobacterium* spp. and *Lactobacillus acidophilus*, as well as the B2 consortium, namely *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* as much as 7.5% (v/v) were inoculated into 250 mL of de Man Rogosa and Sharpe (MRS) media and then incubated at 37°C for 24 hrs. Fresh milk from Koperasi Susu Bandung Utara (KPSBU Lembang) is pasteurized by heating at 70-80°C. Milk that has been heated and then cooled until the temperature of 45°C, then added with 7.5% consortium bacteria, then homogenized. The fermentation or incubation process lasts 14 hrs.

Analysis of lactic acid content and pH of probiotic yogurt: Probiotic yogurt samples were tested for lactic acid content and pH. The probiotic yogurt used in this study was the first (B1) containing *Bifidobacterium* spp.+*L. acidophilus* and the second sample (B2) containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*. Lactic acid content was tested using the titration method and pH was measured using a pH meter starter 3100 M PH and Conductivity Bench production by OHAUS Instrument (Shanghai) Co. Ltd., 7F, Block 33, 680 Guiping Road Shanghai, 200233, China. The lactic acid value is calculated using the following formula:

$$\text{Lactic acid (\%)} = \frac{V_{NaOH} \times N_{NaOH} \times 90}{\text{Volume sample} \times 1000} \times 100$$

Table 1: List of probiotic yoghurt treatments in feed

Category	Treatment
T0	Basal feed only (no probiotics)
T1	Basal feed+2% B1 probiotic powder
T2	Basal feed+3% B1 probiotic powder
T3	Basal feed+2% B2 probiotic powder
T4	Basal feed+3% B2 probiotic powder

B1: *Bifidobacterium* spp.,+ *Lactobacillus acidophilus*, B2: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

Making probiotic powder: In the process of making probiotic powder, probiotic yogurt is added with encapsulant ingredients (skim milk and maltodextrin) then added to sterile distilled water (1/2 of the total volume of solution), then stirred and homogenized. Once homogeneous, the mixture is dried using a spray dryer with an inlet temperature of 160°C and an outlet of 65-70°C to produce probiotic yogurt in powder form.

Experimental procedure

Animal preparation: Forty laying hens, 31 weeks old, female were bred in Sapta Karya Megah and Intama. Laying hens were kept at 24-27°C under a 24 hrs light and 60% relative humidity, with food and water *ad libitum* for 6 weeks in Test Farm Faculty of Animal Husbandry, Universitas Padjadjaran. Laying hens were divided randomly into five group treatments. The control group which was not given the treatment as the control group (P0), group-1 was given treatment with 2% probiotic powder B1 (*Bifidobacterium* spp. and *L. acidophilus*) (P1), group-2 was given treatment by 3% probiotic powder B1 (P2), group-3 was given treatment by 2% probiotic powder B2 (*L. Bulgaricus*, *S. thermophilus*, *L. acidophilus* and *B. bifidum*) (P3), group-4 was given treatment by 3% probiotic powder B2 (P4). All laying hens were habituated for 2 weeks prior to treatments.

Treatments: This treatment was carried out for 6 weeks. Laying hens were given five treatments and repeated eight times so that there were 40 experimental units, with each cage containing 1 laying hen. Each cage was labeled with a treatment and repetition number to facilitate observation and data collection. Probiotic mixed feed was given two times, i.e., in the morning and evening as much as 120 g/head/day and drinking was given *ad libitum*. Places for food and drinking places are always watched and cleaned to prevent disease. The treatments used can be seen in Table 1.

Feed conversion ratio (FCR): In this study, laying hens with the ISA Brown strain aged 31 weeks or who were in the first layer phase were used. Ration conversion calculates the amount of feed needed by chickens to produce one kilogram of egg weight; the calculation method is the cumulative amount of feed consumed divided by the total weight of eggs produced. The remaining feed of the chicken is calculated every day, in addition to the weight of the eggs produced, which is also weighed every day. After that, all data were calculated and tallied at the end of the study.

Hen day production (HDP): Hen day production (HDP) calculates the average number of eggs produced by chickens for one day. The calculation of hen day production is done every day by dividing the number of eggs produced by the number of hens at that time.

Statistical analysis: All data were statistically analyzed by Analysis of Variance (ANOVA) and followed by Duncan's Multiple Range Test using SPSS 22.0. Statistical significance was designated at $p>0.05$.

RESULTS AND DISCUSSION

Lactic acid content and pH of probiotic yogurt: The main principle of the yogurt-making process is fermentation with lactic acid bacteria. This research indicated that probiotic yogurt contains the starter bacteria *Bifidobacterium* spp.+ *L. acidophilus* (B1) has a higher lactic acid content and a lower pH than probiotic yogurt products *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (B2). In B1 yogurt the lactic acid produced is 0.945% with a pH of 4.31. Meanwhile, B2 yogurt has a lower lactic acid content, namely 0.64% with a pH of 4.47.

The microbiological quality of yogurt affects the concentration of lactic acid, such as the content of Bifidobacterium enzyme which converts glucose and galactose into lactic acid. The formation of lactic acid does not inhibit bacterial growth, but lactic acid acts as a growth promoter for LAB itself[8]. The lactic acid bacteria content of probiotic yogurt in this study was in line with previous research which stated that the lactic acid content of *L. acidophilus* and *Bifidobacterium* spp., ranges from 0.81-0.95%[9]. Increasing the concentration of cow's milk and yogurt starter culture resulted in higher lactic acid content and lower pH values[10].

The pH content of probiotic yogurt in this study was in line with research which stated that the pH content of *L. acidophilus* and *Bifidobacterium* spp., ranges from 4.29-4.53[9]. The higher the number of cultures used, the higher the increase in microbial activity and development so that it can increase the rate of breakdown of lactose into lactic acid, thereby reducing the pH value. Lactic acid is inversely

Table 2: Production of laying hens that have been treated with probiotic yogurt

	Group				
Experimental	T0	T1	T2	T3	T4
FCR	2.01[a]	2.10[ab]	2.17[b]	2.08[ab]	1.98[a]
HDP	95.24	95.24	88.10	92.86	89.24
Egg weight	56.05	55.20	51.79	55.89	57.25

FCR: Feed conversion ratio, HDP: Hen day production, Significant[a] = 0.084 and Significant[b] = 0.149

proportional to pH because the greater the amount of lactic acid produced by lactic acid bacteria the lower the pH[11]. Lactic acid bacteria demand nutrients in the medium and generate energy only during the breakdown of carbohydrates. The probiotic and postbiotic potential of lactic acid bacteria from artisanal dairy products against pathogens and found that the antibacterial activity of the LAB cultures resulted from the secretion of organic acids that lowered the pH[12].

Production of laying hens: Ration conversion is a measure used to assess the efficiency of use and quality of rations. A smaller conversion value indicates feed efficiency in egg production and no feed waste. A larger conversion value indicates waste due to the feed not being maximally beneficial to egg production[13]. The results of this study showed that giving probiotic yogurt did not make a real difference to feed conversion ratio (FCR), hen day production (HDP) and egg weight can be seen in Table 2.

The results showed that the administration of probiotic powder had no significant effect on HDP and egg weight, but had a significant on FCR. The different ratio conversion between the control treatment and the treatment using probiotics was caused by the consumption of more rations and the relatively larger weight of eggs causing smaller ratio conversion[14]. In this study, administration of probiotics had a significant effect on FCR but there was an increase in treatment P2 where the FCR value was higher. Thus, the treatment of rations using probiotics is significantly increased because it was in line with the amount of ration consumed and the weight of the eggs. As you can see, the average egg weight of P2 is lower compared to the other treatments. Lactic acid can increase egg-laying speed and improve egg quality due to its probiotic capabilities. This can be caused by the presence of beneficial microorganisms such as lactic acid bacteria which can improve intestinal health and nutrient absorption in chickens. The characteristics of probiotics are persistent in the digestive tract, attach to the epithelium or mucus and compete with the host microflora so they are good for livestock[15]. This is caused by the consumption of more feed and the relatively large weight of the eggs, so the conversion of the feed produced is smaller. In this study, there was only a tendency for a decrease in feed conversion at P4, meaning that the dose in this treatment was optimal.

Many researchers show that giving probiotics to laying hens can improve egg production and increase ration efficiency[16]. Giving probiotics to laying hens can improve egg production, eggshell weight and reduce cholesterol levels in egg yolks[17]. However, in this study, giving probiotics had no significant effect on HDP and egg weight of laying hens. The age of the livestock used in this research was 31 days or 7 months, which is the peak production phase. Research shows that steroid hormones play a role in egg production in chickens. For example, steroid hormones have been shown to regulate the growth of chicken follicle walls, which is important for egg production[18]. Apart from that, at peak production, the genes that influence egg production are in good condition, so even if probiotics are given the effect is not optimal. At the peak of production, the chicken's digestive tract is well formed and has a stable microbiota, so it can be said that probiotics will not have a significant effect on the chicken's digestive tract. Previous research that was in their peak production period also had high production[19]. Egg production is known to have reached its peak if, for 5 consecutive weeks, the percentage of egg production no longer increases. The combination of probiotics had no effect on the performance of laying hens, but a significant increase in albumen crude protein content increased the quality of treated chicken eggs[20].

Further research on the bacteria in probiotic yogurt is needed to find out how the interactions between bacteria, as well as the administration of probiotic powder on ration conversion and total egg production in the final layer phase so that we can know for sure the specific role of these bacteria in inhibiting pathogenic bacteria in livestock.

CONCLUSION

The lactic acid content and pH of B1 (*Bifidobacterium* spp. + *L. acidophilus*) are better than B2 (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*). However, giving probiotic yogurt to laying hens during the peak period did not have a significant effect on ration conversion and production of laying hens.

SIGNIFICANCE STATEMENT

Giving probiotic yogurt powder to chickens in the early egg-laying phase gives different results than giving probiotic yogurt powder to chickens in the final egg-laying phase. This manuscript also showed the probiotic yogurt's lactic acid content and pH. The study showed that giving probiotics to laying hens during their peak period did not have a significant effect because the cells in the chicken's body were in optimal condition. Giving probiotic yogurt in the final phase can improve the hematology of laying hens so that it is good for production. However, providing probiotic yogurt powder to chickens in the early laying phase increased production performance after 2 weeks of treatment, especially in T4.

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