



Increasing Egg Production by Preventing *Salmonella Typhimurium* Infection in Laying Hens with the Addition of Probiotic Yogurt Powder

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Abstract | This study aims to determine the impact of probiotic yogurt on animal health and production. Specifically, it investigates whether and how probiotic yogurt can enhance the productivity of laying hens infected with *S. typhimurium*. The research utilized an experimental method employing a Completely Randomized Design (CRD) with six treatments and five replications. The treatment groups were as follows: T1 was the control group, which did not receive probiotic yogurt and was not infected with *S. typhimurium*; T2 received 4% yogurt probiotics and was not infected; T3 received 6% yogurt probiotics and was not infected; T4, which had no yogurt probiotics, was infected with *S. typhimurium* at a concentration of 10^8 CFU/ml; T5 received 4% yogurt probiotics and was infected; and T6 received 6% yogurt probiotics and was infected. Yogurt probiotic flour was carried out using maltodextrin as an encapsulation method. The preparation of the probiotics followed the procedure: consortium B1 included *Bifidobacterium* spp. and *Lactobacillus acidophilus*, while Consortium B2 comprised *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*. Both consortia were inoculated at a concentration of 7.5% (v/v) into 250 mL of De Man Rogosa and Sharpe (MRS) media and incubated at 37°C for 24 hours. The statistical data were analyzed using Analysis of Variance (ANOVA) and Duncan's multiple range test. In this study, feed conversion ratio (FCR) was measured by comparing the amount of ration consumed with the weight of eggs produced, and hen-day production (HDP) was determined by collecting the number of eggs each day by comparing the number of hens on that day. In contrast, egg weight was determined by weighing the number of eggs produced by each experimental unit. The results indicated that the administration of probiotic yogurt did not significantly affect ($P > 0.05$) the feed conversion ratio (FCR), hen-day production (HDP), egg weight, or consumption in laying hens infected with *Salmonella typhimurium*.

Keywords | Probiotics, Yogurt, Laying hens, *Salmonella typhimurium*, FCR, HDP

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Salmonella infection, especially *Salmonella typhimurium*, is one of the significant challenges in poultry production. This infection can result in reduced chicken performance, such as increased feed conversion ratio (FCR), decreased hen-day production (HDP), and metabolic disorders leading to intestinal inflammation and organ damage, such as the liver. In addition, Salmonella infection has economic consequences due to medical costs, decreased productivity, and food safety risks. However, reports on the prevalence of the disease and its impact on local conditions are limited, and further research is needed (Kanmani and Kim, 2020; Junaid *et al.*, 2024; Rishi *et al.*, 2009).

Probiotics as a non-antibiotic alternative have shown great potential in improving the gastrointestinal health of chickens by reducing Salmonella colonization through mechanisms of nutrient competition, inflammation modulation, and enhancement of local immunity. This study proposes using probiotics to improve feed efficiency and egg production and suppress Salmonella infection, which is an essential step towards creating a sustainable approach in poultry farming. Thus, this research has a strategic position in addressing the challenges of Salmonella infection and improving the sustainability of poultry production systems (Rishi *et al.*, 2009; Junaid *et al.*, 2024).

S. typhimurium infection typically begins with the consumption of pathogens that colonize the intestines and propagate rapidly to internal organs such as the liver and spleen. This infection can negatively impact animal health, leading to a decrease in egg production in laying hens. Transmission of the disease can occur through vector animals and reservoir intermediaries. Given that eggs are a common source of protein for many people, the health of laying hens is crucial. Infected hens may experience deteriorating health and even death. Several studies have indicated that reliable probiotic consortia can inhibit the growth of pathogenic bacteria, including *S. typhimurium* (El-Sharkawy *et al.*, 2020), and inhibit the growth of pathogenic bacteria, including *S. typhimurium* (El-Sharkawy *et al.*, 2020; Manin *et al.*, 2024). Improving sanitation and biosecurity measures in the cage environment is essential to prevent this infection and add health-enhancing animal additives (Pazla *et al.*, 2023). One practical option is probiotics, which contain live microorganisms that promote a healthy digestive tract ecosystem.

Numerous studies have shown that reliable probiotic combinations can produce probiotics from lactic acid bacteria (LAB), which produce primary metabolites such as lactic acid and bacteriocins. These compounds have the potential to act as antimicrobials. Both bacteriocins and bioactive peptides produced during fermentation exhibit properties

that can suppress the activity of pathogenic bacteria. The probiotics employed in this study are reliable, as they are resistant to bile salts and hydrochloric acid (HCl) and are capable of surviving in the large intestine or colon, which is the primary site for pathogenic bacteria (Murry *et al.*, 2004; Abd El-Hack *et al.*, 2020). The findings align with research demonstrating the broader benefits of probiotics, particularly against *S. typhimurium*. Probiotics, such as *Lactobacillus acidophilus* and *Lactobacillus plantarum*, have been shown to enhance gut immunity, modulate inflammation, and maintain intestinal barrier integrity during Salmonella infections. For example, a study found that these probiotics modulate immune responses and improve gut health by regulating cytokine levels and metabolic pathways, which are critical for maintaining gut homeostasis during infections (Kanmani and Kim, 2020; Junaid *et al.*, 2024).

Additionally, probiotics can prevent intestinal damage caused by Salmonella through mechanisms like reducing inflammatory responses, as demonstrated in vitro studies using intestinal epithelial cells (IECs). Probiotics have been found to lower the levels of pro-inflammatory cytokines like IL-8 and TNF- α , which are typically elevated during Salmonella infections (Kanmani and Kim, 2020). Research also emphasizes probiotics' role in alleviating Salmonella's systemic impacts, including liver damage. Probiotic strains can reduce bacterial translocation to the liver and other organs by improving intestinal barrier functions and competitive exclusion of pathogens (Rishi *et al.*, 2009).

The main objective of this research is to investigate how a consortium of bacteria, specifically *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* spp., can enhance the productivity of laying hens infected with *S. typhimurium*. Previous studies have indicated that these bacterial consortia can improve the intestinal ecosystem, promoting animal health, increasing production (Wijayanti and Ardyanti, 2007; Mushawwir *et al.*, 2022), lipid breakdown (Tanuwiria *et al.*, 2022a) and physiological response (Tanuwirian *et al.*, 2022b, 2023).

Conducting this research is crucial to enhancing our understanding of the effects of probiotic yogurt on pathogenic bacteria, especially in monogastric animals. The ultimate aim is to translate these findings for human applications.

MATERIALS AND METHODS

This research was conducted between October and November 2024 at the Biochemistry and Food Chemistry Laboratory, Ciparanje Faculty of Animal Husbandry, Padjadjaran University.

PREPARING PROBIOTIC YOGURT POWDER

The bacteria used in this study were sourced from the Nano-

bio Laboratory. The entire preparation process was standardized and based on [Adriani et al. \(2024a\)](#) and [Firmansyah et al. \(2024\)](#). Two bacterial consortia were employed, designated as B1 and B2. Consortium B1 included *Bifidobacterium* spp. and *Lactobacillus acidophilus*, while Consortium B2 comprised *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*. All combinations of these bacteria are known to interact without competing, and these bacteria produce the most optimal lactic acid. Both consortia were inoculated at a concentration of 7.5% (v/v) into 250 mL of De Man Rogosa and Sharpe (MRS) media and incubated at 37°C for 24 hours. Meanwhile, 1 liter of fresh milk from the North Bandung Milk Cooperative (KSBU Lembang) was pasteurized at 80°C. After cooling the milk to 45°C, 7.5% of the bacterial consortia was added and homogenized. The fermentation process lasted for 14 hours. The probiotic yogurt was mixed with an encapsulating agent (maltodextrin) and then added to sterile distilled water, constituting half the total solution volume to produce the probiotic powder. The mixture was stirred and homogenized until uniform. Finally, the homogeneous mixture was dried using a spray dryer with an inlet temperature of 160°C and an outlet temperature of 65-70°C, resulting in probiotic yogurt in powder form. In simple terms, the procedure for making probiotic flour is shown in [Figure 1](#).

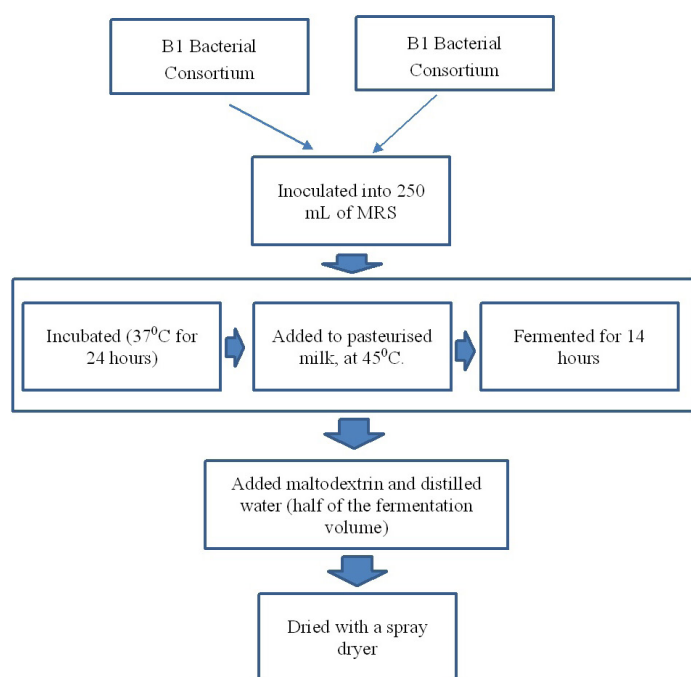


Figure 1: Stages of probiotic flour preparation.

PREPARING *S. TYPHIMURIUM*

Bacterial isolate *Salmonella enterica* sv Typhimurium strain code ATCC-14028 was selected for use in this study. Before use, the bacteria were resuscitated by streaking them on nutrient agar. To generate a challenge inoculum, one colony of *S. Typhimurium* was added to five ml of Luria Bertani

(LB) broth and incubated at 37°C with shaking (110 rpm) for 6 hours. Next, ten µl of this culture was added to 10 ml of fresh LB broth and incubated under the same conditions overnight. The number of bacteria in the broth was determined by measuring the optical density at 600 nm using a spectrophotometer. Bacteria were diluted in LB to reach the desired concentration. A challenge dose of 1×10^8 CFU *S. Typhimurium* was selected for this study based on considerations of previous research.

At 26 weeks of age, 15 chickens from each group were orally gavaged with 1×10^8 CFU of *S. Typhimurium* in a 1.0 mL inoculum. During the first 48 hours post-infection, chickens were monitored twice daily, focusing on feed consumption, the appearance of clinical signs following the *S. typhimurium* challenge, and eventual mortality (McWhorter and Chousalkar, 2018). This inoculation process may be performed more than once (repeat inoculation), which may help to establish colonization and strengthen the local immune response in the gut ([Khan et al., 2024](#)).

EXPERIMENTAL PROCEDURES

ANIMAL SAMPLE: Thirty laying hens, 26 weeks old, were maintained at 24-27 °C under continuous light for 24 hours and 60% relative humidity. Temperature and humidity are continuously recorded using a digital thermometer and hygrometer. They had ad libitum access to feed and drinking water for six weeks at the Ciparanje Farm, Faculty of Animal Husbandry, Padjadjaran University. The hens were randomly divided into six treatment groups: Control Group T1: No yogurt probiotics and not infected with *S. typhimurium*. Group T2: 4% yogurt probiotics and not infected with *S. typhimurium*. Group T3: 6% yogurt probiotics and not infected with *S. typhimurium*. Group T4: No yogurt probiotics and infected with *S. typhimurium* at a 10^8 CFU/ml concentration. Group T5: 4% yogurt probiotics and infected with *S. typhimurium*. Group T6: 6% yogurt probiotics and infected with *S. typhimurium*. The laying hens were allowed a two-week adaptation period before treatment commenced.

TREATMENT: The treatment was conducted over 6 weeks. Laying hens receiving probiotic powdered yogurt were subjected to seven different therapies, and each was repeated five times. Each cage housed one laying hen and was labeled with treatment and repetition numbers to facilitate observation and data collection. The hens were fed a probiotic mixed feed twice daily—once in the morning and once in the evening—at a rate of 120 grams per head per day, with unlimited access to water. The feeding and drinking areas were regularly monitored and cleaned to prevent the spread of disease. A summary of the treatments used can be found in [Table 1](#).

FEED CONVERSION RATIO (FCR): This study used Lohmann Brown strain laying hens that were 26 weeks old or in

their first laying phase. Ration conversion measures the feed required for the chickens to produce one kilogram of egg weight. This is calculated by dividing the cumulative amount of feed consumed by the total weight of the eggs produced. Each day, we recorded the remaining feed and the eggs' weight. At the end of the study, all data were compiled and tallied for analysis.

Table 1: List of probiotic yogurt treatments in feed.

Category	Treatment
T1	Only basal feed without bacterial pathogen
T2	Basal feed + 4% yogurt probiotics, without bacterial pathogen
T3	Basal feed + 6% yogurt probiotics, without bacterial pathogen
T4	Only basal feed with the bacterial pathogen
T5	Basal feed + 4% yogurt probiotics with bacterial pathogen
T6	Basal feed + 6% yogurt probiotics with bacterial pathogen

HEN DAY PRODUCTION (HDP): HDP measures the average number of eggs chickens produce in one day. To calculate HDP, you divide the total number of eggs produced by the number of hens present at that time, and this calculation is performed daily.

STATISTICAL ANALYSIS

All data were statistically analyzed using Analysis of Variance (ANOVA) followed by Duncan's multiple range test in SPSS version 22.0. Statistical significance was set at $P < 0.05$. The relationship between FCR and HDP was analyzed using univariate correlation regression.

RESULTS AND DISCUSSION

This study's findings, shown in Table 2, indicated that administering probiotic yogurt did not affect the FCR, HDP, egg weight, or consumption.

The results of this study indicate that the administration of probiotic yogurt did not show any significant differences from the ANOVA test. However, probiotic yogurt did not show a significant overall effect ($P > 0.05$), and probiotic yogurt showed potential benefits at a dose of 4%. Specifically, this dosage could reduce the FCR, increase HDP, and increase egg weight. In the current study, it appears that the Salmonella treatment (T4 - T6) showed no ($P > 0.05$) change in performance, even without the addition of probiotic yogurt (T4). However, in this experiment, the group of chickens fed with 4% probiotic yogurt, without Salmonella (T2), showed a better performance improvement trend than the other groups of chickens. The results of the analyses showing that the treatments in this study did not

have a significant effect ($P > 0.05$) could be due to the inadequate number of experimental chickens, even though the experimental chickens were selected to be homogeneous regarding genetics, age, and body weight. The FCR values were generally lower in the groups receiving probiotics at specific doses (T2 to T5) than in the control group (T1). The lowest FCR value was found in group T5, at 1.81, indicating better feed utilization efficiency. However, in group T6, which received a higher dose of probiotics, the FCR increased significantly to 2.39, suggesting potential adverse effects from excessive probiotic doses.

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

Experimental Group	Group					
	T1	T2	T3	T4	T5	T6
FCR	1.97	1.83	1.92	1.91	1.81	2.39
Egg Weight	56.68	57.00	53.61	55.89	56.39	49.15
Consumption	3416.60	3201.0	3173.80	3272.20	3161.80	3400.40

FCR: Feed conversion ratio.

The differences in feed conversion ratios between the control group and the group given probiotics were likely due to higher feed consumption and the relatively more considerable egg weight in the probiotic group, which led to a lower feed conversion ratio (Xiang *et al.*, 2019; Tanuwiria *et al.*, 2022b). Additionally, the study found that chickens exposed to *S. typhimurium* had lower production rates than those not exposed to the bacteria (Figure 2). This is illustrated in the picture below.

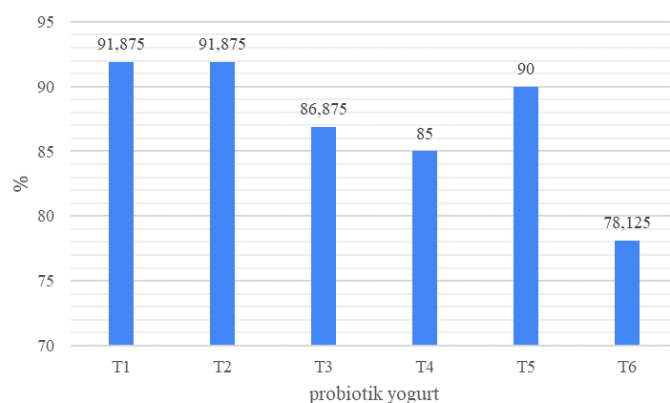


Figure 2: Hen day production.

Daily chicken production fluctuated (Figure 2). Group T1 and T2 achieved the highest HDP value at 91.875, respectively, while T6 significantly declined to 78.13. This decrease may be attributed to *S. typhimurium*'s impact on the laying hens' health, thereby reducing their productivity. In the T5 treatment, egg weight was similar to that of the control group (56.39 g vs. 56.68 g), while the T6 group

recorded the lowest egg weight at 49.15 g. This indicates that administering probiotic doses up to 4% (T5) can help maintain or slightly increase egg weight in chickens exposed to *S. typhimurium*; however, higher doses may lead to reduced productivity.

Results show that the lower the FCR value (meaning better feed efficiency), the higher the daily egg production. In other words, if chickens convert feed into energy more efficiently, they will produce more eggs. In contrast, egg production decreases when FCR increases (less efficient feed) (Figure 3). The strong R^2 highlights that FCR is a significant predictor of HDP ($P < 0.05$), emphasizing the importance of efficient feed management in poultry farming. The negative slope of the regression line indicates an inverse relationship: as FCR increases (indicating less feed efficiency), HDP decreases. This is expected since lower efficiency in converting feed to energy negatively impacts egg production. The clustering of data points near the regression line further supports the strength of this relationship. The correlation regression results showed an increase followed by high ratio efficiency (FCR) in HDP.

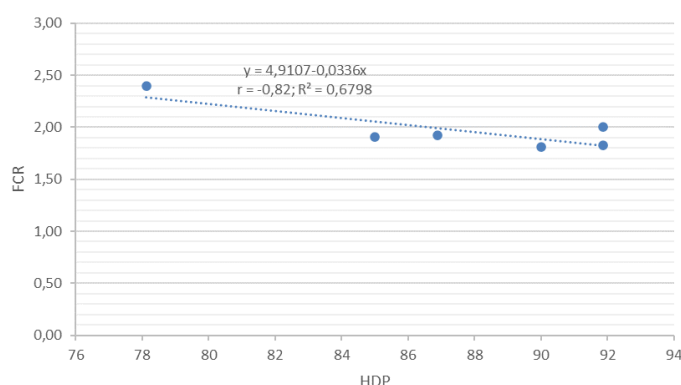


Figure 3: Correlation between FCR and HDP.

The regression analysis shows the index of determination (R^2) for the effect of FCR on HDP (Figure 3), which is $R^2 = 0.6798$; this indicates that the decrease in HDP is influenced by FCR by 67.98%; the rest is the impact of other factors (Figure 3). This means that the higher the nutrient utilization inefficiency, the higher the reduction in HDP, which based on the relationship between these two variables, in the results of the study, this inefficiency was able to influence 67.98% of the reduction in egg production. Figure 2 also shows the regression equation of $y = 4.9107 - 0.0336x$, meaning that each increase or decrease in ratio efficiency by one unit causes a reduction in HDP by 0.0336%.

While FCR increased from 1.81 (at T5) to 2.39 (at T6), HDP dropped from 90.00 to 78.13. This shows that feed efficiency significantly affects production yield. A lower FCR indicates that the hens can feed more optimally for growth and egg production. The probiotics may have

helped improve feed efficiency, especially at specific doses (such as in T5). This explains why HDP was higher in the low FCR treatment.

S. typhimurium infection in the digestive tract of chickens can cause intestinal inflammation (enteritis) and impair nutrient absorption. As a result, FCR increases as chickens need more feed to produce the same energy. The infection also causes physiological stress that can lower HDP. Infected hens usually show reduced egg production, lower egg quality, and increased mortality risk if the infection becomes systemic. The study showed that hens infected with *S. typhimurium* had poorer FCR than healthy hens, with reduced body weight and egg productivity. Salmonella infection increases tumor-induced factor- $\kappa\beta$ (TIF- $\kappa\beta$) levels. It is known that TIF- $\kappa\beta$ stimulates inflammation, along with proinflammatory interleukins (Xu *et al.*, 2023). Other researchers have shown that TIF- $\kappa\beta$ also induces an increase in proinflammatory hormones (Xiang *et al.*, 2019) and also raises the rate of formation of non-carbohydrate precursors (lipids and amino acids) into glucose, pyruvate, and energy, an essential reason for the decline in immune function.

When probiotics are administered, as in this study (optimal dose T5), they likely help reduce the population of pathogens such as *S. typhimurium*. With less disruption in the digestive tract, chickens can utilize nutrients better, resulting in lower FCR and higher HDP. In addition, chickens free from Salmonella tend to be in better health, resulting in optimal egg production. Probiotics with this consortium have high protease enzymes, so giving this probiotic yogurt can affect the egg formation process in laying hens (Situmeang *et al.*, 2024) and vitellogenin gene expression (Dudi *et al.*, 2023).

In group T5, feed consumption was recorded at 3161.80 g, lower than the control group T1, which consumed 3416.60 g. This indicates that the probiotics used at this dosage can enhance feed efficiency without increasing overall consumption. In contrast, feed consumption in group T6 increased to 3400.40 g, which aligns with the higher FCR observed in that group. The results indicate that administering probiotics yogurt at a dose of up to 4% (T5) yields the best outcomes regarding reducing FCR, increasing HDP, and maintaining egg weight. However, a higher dosage of probiotics (T6) may negatively impact the production performance of laying hens, as evidenced by an increase in FCR and a decrease in both HDP and egg weight. Previous studies have also shown that probiotics do not significantly affect laying hens' feed conversion or egg weight (Adriani *et al.*, 2024a; Nurfauziah *et al.*, 2024). Feeding 6% yogurt probiotics, also infected with salmonella, did not increase production. This result may be because the 6% level appears too high, resulting in acidic conditions in the ileum that damage the chemical structure of nutrients.

It is crucial to use probiotic yogurt as a supplement with care, paying close attention to the optimal dosage to maximize benefits without negatively affecting production performance. Previous studies have shown that probiotic supplementation can significantly increase egg production and weight, particularly with strains such as *Lactobacillus* and *Bacillus*. The bacteria in probiotic yogurt produce lactic acid and bacteriocins, which contribute to improved animal health (Kharazi *et al.*, 2022; Mushawwir *et al.*, 2022; Rahmania *et al.*, 2022). This positive impact has been associated with better production performance in laying hens (Alaqil *et al.*, 2020; Aritonang *et al.*, 2024).

Probiotics, especially those derived from *Lactobacillus* and *Bacillus subtilis* strains, can inhibit the colonization of *Salmonella* in chickens' digestive tracts by competing for attachment sites on the intestinal mucosa and nutrients. This competition helps to maintain a healthy gut microbiota and reduces the risk of *Salmonella* infections. One study found that probiotic supplementation in laying hens enhanced gut health (Mushawwir *et al.*, 2021a,b; Purwanti *et al.*, 2024) and decreased the prevalence of *Salmonella* enteritidis in the digestive system. This positively impacts egg quality and food safety, especially since eggs can be a source of *Salmonella* contamination (Xu *et al.*, 2023; Firmansyah *et al.*, 2024).

Probiotics produce organic acids, such as lactic acid, which lower intestinal pH, making the environment less favorable for *Salmonella* growth. Additionally, specific probiotic strains are known to produce bacteriocins, which possess specific antimicrobial properties (Getachew, 2016; Manin *et al.*, 2024). In addition, the provision of probiotic yogurt has also been shown to positively affect the immunity of livestock and increase productivity even under heat-stress conditions (Adriani *et al.*, 2024b,c). The presence of *Salmonella* in the digestive tract stimulates cytokinin signaling. The higher the *Salmonella* population, the higher the cytokinin levels in the blood. Cytokinin provides positive feedback to the increase of glucocorticosterone or glucocorticoids (Mushawwir *et al.*, 2024; Tanuwiria *et al.*, 2023). This hormone causes an increase in energy production activity through the gluconeogenesis pathway (Mushawwir *et al.*, 2022), causing the mobilization of fatty acids (Muhammad *et al.*, 2023) and amino acids into the gluconeogenesis cycle. This phenomenon may be an essential reason for reducing precursors in egg formation and raising inflammation—the rate of vitellogenesis or biosynthesis of egg precursor formation, resulting in decreased egg production.

This research indicates that probiotics can improve FCR and HDP when administered at optimal doses (T5). This improvement is likely due to probiotics' ability to promote gut health, which may also help suppress the colonization of pathogens such as *Salmonella*. By maintaining gut in-

tegrity and reducing the prevalence of *Salmonella*, probiotics can directly increase chicken productivity and improve egg quality, making them safer for consumption. Additionally, *Salmonella* causes increased inflammation in ileal cells, which is induced by cytokines (pro-inflammatory substances). Heightened inflammation and cell death can reduce the capacity of villi to absorb nutrients, ultimately leading to increased FCR or decreased ratio efficiency.

CONCLUSIONS AND RECOMMENDATIONS

In this study, probiotics did not demonstrate a significant impact on the productivity of laying hens. However, using probiotics to manage these hens improves feed efficiency and egg production. In addition, probiotics can serve as an effective biosecurity strategy against *Salmonella*. The results showed that chickens infected with *Salmonella* maintained stable productivity. This means that probiotics could create a more acidic ileal ecosystem, so pathogenic bacteria could not survive. The 4% dose (T4) optimally balanced feed efficiency, production, and egg quality, making it the most effective dose in this study. Using probiotics has excellent opportunities to be applied from the beginning of the rearing period by adjusting the feeding level. Further research is needed to explore the relationship between probiotic dose, gut health, and *Salmonella* reduction, particularly in laying hens.

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NOVELTY STATEMENTS

This study is one of the few evaluating the potential of probiotic yogurt to mitigate the adverse effects of *S. typhimurium* infection in laying hens. It provides a targeted approach to improving poultry health through functional food supplementation. The study utilizes maltodextrin-based encapsulation for probiotic yogurt, an innovative technique to ensure probiotics' stability and viability during the gastrointestinal passage, which is not commonly applied in poultry research.

AUTHOR'S CONTRIBUTIONS

The author has done the research and writing of this article with the contribution of Collecting data, data analysis, and

writing the manuscript (ASR); Collecting data and data analysis (MAZ); Assembling research design and reviewing the manuscript (LA); Data analysis and review manuscript (AM)

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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