

## EFFECTS OF FREEZE-DRIED PROBIOTIC AND PARABIOTIC *LACTOBACILLUS* SPP. ON GROWTH PERFORMANCE AND FECAL MICROFLORA OF BROILERS UNDER HEAT STRESS CONDITIONS

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### Abstract

*This study evaluated the effects of freeze-dried probiotic and parabiotic Lactobacillus spp. on growth performance and fecal microflora of broiler chickens reared under heat stress conditions (32–39°C). A total of 252 day-old Cobb broilers were randomly assigned to six treatments, including probiotic and parabiotic Lactobacillus fermentum I25 and Lactobacillus brevis C10, positive control with Zn bacitracin (+), and negative control (-). Growth performance was recorded weekly, and fecal microflora was analyzed at weeks 2 and 6. Results showed that supplementation, particularly parabiotic Lactobacillus brevis C10, significantly improved body weight and average daily gain at later stages ( $p < 0.05$ ). Lactic acid bacteria populations increased significantly, while Enterobacteriaceae were unaffected. These findings suggest that parabiotics may serve as a stable alternative to probiotics under heat stress conditions, with potential application as antibiotic growth promoter substitutes in poultry production.*

*Keywords: Lactobacillus, poultry, probiotics*

### Introduction

In recent years, the use of probiotics as an alternative therapeutic approach has garnered significant attention. One common method for producing probiotic preparations in dried powder form is freeze-drying (Ge et al., 2024). Optimizing the production process of such probiotic formulations is crucial to obtain products with desirable properties and higher numbers of

viable probiotic microorganisms. Among the various probiotic preparations, there has been a growing interest in developing dried formulations (Kieps and Dembczyński, 2022).

*Lactobacillus* sp. has demonstrated the potential to induce a protective response against gastrointestinal infection agents in rats when orally administered (Hrdý et al., 2022). This response is associated with the synthesis of anti-inflammatory cytokines such as IFN-IL, IL-2, IL-6, TNF- $\alpha$ , and nitric oxide (Oladejo et al., 2023). Many probiotic strains of *Lactobacillus* produce various antibacterial compounds, including lactacin B, lactacin F, nisin, and reuterin (3-hydroxypropionaldehyde), a broad-spectrum antibiotic that is active against bacteria, yeast, fungi, protozoa, and viruses (Latif et al., 2023).

Probiotics play a crucial role in balancing the ecosystem in the digestive tract and exhibit antimicrobial activity by breaking down proteins, thereby increasing fecal ammonia levels (Wang et al., 2021). *Lactobacillus* sp. contributes to this antimicrobial effect by producing organic acids, particularly lactic acid, through its metabolic processes, which act against *E. coli* (Hudson et al., 2020). Additionally, *Lactobacillus* sp. produces a bacteriocin known as plantaricin ASM-1, which can inhibit the growth of *Escherichia coli* (Main et al., 2020). Bacteriocins function by forming cavities or pores in the cell walls/membranes of sensitive microbes and creating potential pH gradients, leading to cellular damage and lysis (Zhang et al., 2016). However, comparative studies evaluating the effectiveness of freeze-dried probiotics and parabiotics, particularly under high ambient temperature conditions typical of tropical environments, are still limited.

Therefore, this study aimed to evaluate the effects of feeding freeze-dried probiotic and parabiotic *Lactobacillus* sp. (*Lactobacillus fermentum* I25 and *Lactobacillus brevis* C10) on the growth performance and fecal microflora of broiler chickens reared under heat stress conditions, with the hypothesis that such supplementation would enhance growth performance and beneficially modulate fecal microflora by increasing lactic acid bacteria populations.

## Material and methods

### Ethic approval

The study complied with animal welfare and ethic regulations under Indonesian Law No. 18/2009 regarding Animal Husbandry and Health, and its amendment Law No. 41/2014. The research adhered to national guidelines ensuring proper housing, continuous access to food and water, and appropriate environmental management to minimize animal stress and promote welfare during the experimental period.

### Birds and experimental facility

The study was conducted at a commercial broiler farm situated in Jombang, East Java, Indonesia, at an altitude of approximately 350 m. For this research, a total of 252 day-old chicks (DOC) from the Cobb strain were selected, each with an average body weight of 56.8 grams. The chicks were randomly allocated into 36 pens, with each pen consisting of 7 chicks. These samples were further divided into six groups, each comprising six pens. The groups were as follows: freeze-dried *Lactobacillus fermentum* I25 (FDI25), parabiotic *Lactobacillus fermentum* I25 (PI25), freeze-dried *Lactobacillus brevis* C10 (FDC10), parabiotic *Lactobacillus brevis* C10 (PC10), positive control with Zn bacitracin (+), and negative control (-). All pens were initially standardized for weight.

To ensure optimal conditions for the chicks' growth, all cages were equipped with drinkers and feeders. Throughout the experiment, the chicks were provided with *ad libitum* access to both feed and water. Additionally, the lighting schedule was maintained at 24 hours of continuous light during the first week, followed by a 12-hour light and 12-hour dark cycle

for the remaining duration of the study. The temperature inside the cages varied throughout the experimental period, ranging from 32°C in the early phase to a peak of 39°C during the hottest period, while relative humidity was maintained between 49% and 69%. These controlled environmental conditions aimed to provide a conducive and consistent setting for the broiler chickens' development and health throughout the research period.

### Treatments

The experimental diet utilized in this study was formulated based on a combination of corn and soybean meal and tailored specifically for the starter (1–3 weeks of age) and grower (3–6 weeks of age) stages of the broiler chickens (Table 1). The feed was provided in the form of mash, which is finely ground to facilitate easy consumption and digestion by the birds. To enhance the nutritional profile and potential health benefits, probiotic *Lactobacillus* sp. was supplemented at a concentration of 1.73% during the starter phase and 2.43% during the growing phase of the broilers.

The nutritional composition of the feed was as follows: crude protein content ranged from 16.0% to 22.2%, crude fiber content was between 8.2% and 8.5%, crude fat content ranged from 8.3% to 8.4%, and ash content varied from 9.3% to 13.3%. Additionally, the feed was analyzed for BETN (digestible amino acid profile) with a range of 41.3% to 43.0% and ME (metabolizable energy content) with a range of 2743.6 to 2872.8 kcal/kg. Ingredients and composition of the broiler diets can be seen in Table 1. These nutritional values were carefully considered to ensure that the broiler chickens received a balanced and appropriate diet that could support their growth, development, and overall well-being throughout the different stages of their life cycle.

Table 1. Ingredients and composition of the broiler diets

| Item (% unless noted)        | Starter | Grower  |
|------------------------------|---------|---------|
| Corn                         | 48.00   | 57.00   |
| Soybean                      | 42.00   | 32.00   |
| Wheat pollard                | 0.00    | 3.00    |
| Palm oil                     | 6.00    | 3.00    |
| Dicalcium phosphate          | 2.00    | 2.00    |
| Limestone                    | 1.00    | 1.00    |
| Premix                       | 2.00    | 2.00    |
| Nutritional composition (%): |         |         |
| Dry matter                   | 89.52   | 88.32   |
| Crude protein                | 16.01   | 22.24   |
| Crude fiber                  | 8.53    | 7.23    |
| Crude fat                    | 8.40    | 8.25    |
| Ash                          | 13.29   | 9.25    |
| BETN                         | 43.07   | 41.32   |
| ME (Kcal.Kg)                 | 2743.56 | 2872.84 |

Abbreviations: BETN, nitrogen-free extract; ME, metabolizable energy.

### Measurement

Growth performance parameters, including feed intake, body weight, average daily gain (ADG), and feed conversion ratio (FCR), were recorded weekly over a 42-day experimental period. Feed intake was calculated as the difference between feed offered and feed refused, while FCR was calculated as the ratio of feed intake to body weight gain.

Fecal samples were collected from each pen at weeks 2 and 6. Approximately 10 g of fresh fecal samples were aseptically collected, pooled per pen, and immediately transported to the laboratory for analysis. Microbial populations were determined using standard plate count methods. Enterobacteriaceae were enumerated on MacConkey agar, while lactic acid bacteria (LAB) were cultured on de Man, Rogosa, and Sharpe (MRS) agar. Plates were incubated at 37°C for 24–48 h, and results were expressed as log<sub>10</sub> colony-forming units per gram (CFU/g) of feces.

## Statistical analysis

The experiment was arranged in a completely randomized design (CRD) with six dietary treatments and six replicates per treatment, with each pen (n=7 birds) considered as the experimental unit. Data were subjected to one-way analysis of variance (ANOVA) using SPSS (IBM Corp., Armonk, NY, USA). Prior to analysis, data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene’s test). When significant differences among treatments were detected ( $p<0.05$ ), means were separated using Duncan’s multiple range test.

## Results

Growth performance and fecal microflora of broiler chickens fed with probiotic *Lactobacillus fermentum/brevis* are presented in Table 2 and Table 3. No significant differences ( $p>0.05$ ) in growth performance parameters were observed during the first two weeks across all treatment groups, indicating a similar initial adaptation period. However, treatment effects began to emerge from week 3 onward. At week 3, ADG was significantly higher in the FDC10 group (27.1 g) compared to the control (+) group (19.4 g) ( $p=0.044$ ). This improvement became more pronounced at week 5, where P125 (78.9 g) and FDC10 (79.9 g) exhibited significantly higher ADG than the control (-) (49.3 g) ( $p=0.003$ ).

By week 6, all growth performance parameters were significantly influenced by dietary treatments, including feed intake ( $p<0.001$ ), body weight ( $p=0.034$ ), ADG ( $p=0.034$ ), and FCR ( $p=0.004$ ). The highest body weight was recorded in FDC10 (1757.6 g) and PC10 (1760.9 g), while the lowest was observed in the negative control group (1491.9 g). The best FCR was found in the positive control group (1.14). Despite these improvements, the overall body weight at week 6 (1491.9–1760.9 g) tended to be lower than typical commercial standards for broiler chickens, which may be associated with environmental conditions during the rearing period, particularly the relatively high ambient temperature (32–39°C).

In terms of fecal microflora, the population of Enterobacteriaceae was not significantly affected by the treatments ( $p>0.05$ ) at both observation times. In contrast, LAB populations increased significantly at weeks 2 and 6 ( $p<0.001$ ). At week 2, higher LAB counts were observed in FDC10 and PC10 groups compared to controls. This trend continued at week 6, where the highest LAB population was recorded in PC10 ( $7.63 \times 10^3$  CFU/g), followed by FDC10 ( $3.82 \times 10^3$  CFU/g). These results indicate that supplementation with probiotic and parabiotic *Lactobacillus* sp. effectively enhanced beneficial gut microbiota in broiler chickens.

Table 2. Growth performance of broiler chickens fed with probiotic *Lactobacillus fermentum/brevis*

| Parameters     | Control (-)        | Control (+)       | FDI25              | P125               | FDC10             | PC10               | Pooled SEM | Sig.  |
|----------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------------------|------------|-------|
| <b>Week 1</b>  |                    |                   |                    |                    |                   |                    |            |       |
| Feed intake, g | 19.4               | 22.1              | 18.3               | 20.7               | 23.7              | 19.0               | 1.19       | 0.799 |
| Body weight, g | 82.0               | 81.0              | 76.4               | 88.4               | 91.3              | 85.1               | 3.59       | 0.883 |
| ADG, g         | 11.9               | 11.7              | 11                 | 12.7               | 12.9              | 12.1               | 0.50       | 0.922 |
| FCR            | 1.57               | 2.00              | 1.71               | 1.57               | 1.86              | 1.43               | 0.079      | 0.337 |
| <b>Week 2</b>  |                    |                   |                    |                    |                   |                    |            |       |
| Feed intake, g | 39.6               | 50.4              | 47.1               | 43.4               | 50.1              | 39.9               | 3.01       | 0.842 |
| Body weight, g | 186.6              | 183.7             | 167.1              | 207.4              | 215.9             | 187.6              | 7.39       | 0.472 |
| ADG, g         | 15.0               | 14.6              | 13.0               | 17.0               | 17.9              | 14.6               | 0.58       | 0.166 |
| FCR            | 2.57               | 3.28              | 3.42               | 2.57               | 2.71              | 2.57               | 0.147      | 0.324 |
| <b>Week 3</b>  |                    |                   |                    |                    |                   |                    |            |       |
| Feed intake, g | 56.4               | 68.1              | 55.4               | 65.3               | 72.1              | 76.7               | 3.16       | 0.312 |
| Body weight, g | 328.3              | 320.1             | 327.6              | 359.4              | 406.1             | 351.7              | 9.66       | 0.094 |
| ADG, g         | 20.3 <sup>ab</sup> | 19.4 <sup>b</sup> | 22.7 <sup>ab</sup> | 21.6 <sup>ab</sup> | 27.1 <sup>a</sup> | 23.4 <sup>ab</sup> | 0.76       | 0.044 |
| FCR            | 2.86               | 3.43              | 2.43               | 3.29               | 2.71              | 3.29               | 0.166      | 0.480 |

|                |                     |                      |                     |                      |                     |                     |       |        |
|----------------|---------------------|----------------------|---------------------|----------------------|---------------------|---------------------|-------|--------|
| <b>Week 4</b>  |                     |                      |                     |                      |                     |                     |       |        |
| Feed intake, g | 86.7                | 95.7                 | 80.0                | 92.1                 | 104.3               | 112.9               | 12.38 | 0.514  |
| Body weight, g | 561.9               | 518.3                | 543.9               | 588.3                | 703.1               | 607.9               | 19.56 | 0.090  |
| ADG, g         | 33.4                | 28.1                 | 31.1                | 32.7                 | 42.4                | 36.4                | 2.34  | 0.617  |
| FCR            | 2.86                | 4.00                 | 3.14                | 2.86                 | 2.57                | 3.57                | 0.106 | 0.079  |
| <b>Week 5</b>  |                     |                      |                     |                      |                     |                     |       |        |
| Feed intake, g | 96.6                | 91.3                 | 93.7                | 123.6                | 115.6               | 95.4                | 4.14  | 0.109  |
| Body weight, g | 906.0               | 892.0                | 950.0               | 1140.0               | 1265.0              | 1119.0              | 21.59 | 0.345  |
| ADG, g         | 49.3 <sup>b</sup>   | 53.3 <sup>ab</sup>   | 58.0 <sup>ab</sup>  | 78.9 <sup>a</sup>    | 79.9 <sup>a</sup>   | 73.0 <sup>ab</sup>  | 3.12  | 0.003  |
| FCR            | 2.14                | 1.71                 | 1.57                | 1.57                 | 1.42                | 1.57                | 0.105 | 0.479  |
| <b>Week 6</b>  |                     |                      |                     |                      |                     |                     |       |        |
| Feed intake, g | 131.9 <sup>bc</sup> | 137.6 <sup>bc</sup>  | 113.9 <sup>b</sup>  | 152.7 <sup>a</sup>   | 129.3 <sup>bc</sup> | 76.0 <sup>c</sup>   | 4.50  | <0.001 |
| Body weight, g | 1491.9 <sup>b</sup> | 1655.4 <sup>ab</sup> | 1551. <sup>ab</sup> | 1595.4 <sup>ab</sup> | 1757.6 <sup>a</sup> | 1760.9 <sup>a</sup> | 29.82 | 0.034  |
| ADG, g         | 83.7 <sup>ab</sup>  | 108.9 <sup>a</sup>   | 86.0 <sup>ab</sup>  | 65.1 <sup>b</sup>    | 70.3 <sup>ab</sup>  | 91.9 <sup>ab</sup>  | 4.25  | 0.034  |
| FCR            | 1.71 <sup>ab</sup>  | 1.14 <sup>b</sup>    | 1.57 <sup>ab</sup>  | 2.85 <sup>a</sup>    | 1.85 <sup>ab</sup>  | 1.85 <sup>ab</sup>  | 0.162 | 0.004  |

Abbreviations: ADG, average daily gain; FCR, feed conversion ratio; SEM, standard error of the mean; Sig., significance (p-value); FD, freeze-dried probiotic; P, parabiotic; Control (–), negative control; Control (+), positive control. Different superscripts within the same row indicate significant differences (p<0.05).

Table 3. Fecal microflora of broiler chickens fed with probiotic *Lactobacillus fermentum/brevis*

| Parameters                                    | Control (–)       | Control (+)       | FDI25             | P125               | FDC10              | PC10              | Pooled SEM | Sig.   |
|-----------------------------------------------|-------------------|-------------------|-------------------|--------------------|--------------------|-------------------|------------|--------|
| <b>Fecal microflora (Week 2)</b>              |                   |                   |                   |                    |                    |                   |            |        |
| Enterobacteriaceae (×10 <sup>9</sup> CFU/g)   | 2.70              | 7.73              | 3.40              | 2.37               | 0.83               | 0.57              | 1.260      | 0.681  |
| Lactic acid bacteria (×10 <sup>3</sup> CFU/g) | 0 <sup>b</sup>    | 0 <sup>b</sup>    | 0.23 <sup>b</sup> | 0.96 <sup>ab</sup> | 1.90 <sup>a</sup>  | 1.83 <sup>a</sup> | 0.218      | <0.001 |
| <b>Fecal microflora (Week 6)</b>              |                   |                   |                   |                    |                    |                   |            |        |
| Enterobacteriaceae (×10 <sup>9</sup> CFU/g)   | 0.42              | 0.21              | 1.09              | 0.03               | 1.14               | 0.50              | 0.181      | 0.413  |
| Lactic acid bacteria (×10 <sup>3</sup> CFU/g) | 0.01 <sup>b</sup> | 0.09 <sup>b</sup> | 0.62 <sup>b</sup> | 0.40 <sup>b</sup>  | 3.82 <sup>ab</sup> | 7.63 <sup>a</sup> | 0.755      | <0.001 |

Means within a row without common superscript are significantly different at p<0.05.

## Discussion

The present study investigated the impact of feeding freeze-dried probiotic and parabiotic *Lactobacillus* sp. on the growth performance of broiler chickens. Notably, the inclusion of probiotics in the feed significantly influenced parameters such as feed intake, body weight, and FCR. This effect can be attributed to the antimicrobial activity of metabolites produced by *Lactobacillus* sp., which inhibit the growth of gut pathogens and promote improved villi height and crypt depth in the intestines of layer hens and broilers (Dempsey and Corr, 2022). Moreover, the use of cryoprotectants, such as trehalose, has been reported to enhance the survival of *Lactobacillus salivarius* during freeze-drying and storage (Majidzadeh Heravi et al., 2022). Such findings underscore the potential benefits of probiotic supplementation in improving gut health and nutrient absorption in poultry.

Despite these improvements, the body weight achieved at week 6 (1491.9–1760.9 g) was relatively lower than standard commercial targets for broiler chickens. This discrepancy may be associated with environmental stress, particularly the high ambient temperature (32–39°C) recorded during the rearing period. Heat stress is known to negatively affect broiler performance by reducing feed intake, altering metabolic processes, and impairing nutrient utilization efficiency. Under such conditions, broilers tend to divert energy from growth toward thermoregulation, which ultimately limits body weight gain (Jongbo et al., 2026). Therefore, although probiotic and parabiotic supplementation improved growth performance compared to controls, the overall performance may not have reached its optimal potential due to thermal stress.

The examination of broiler chicken fecal microflora revealed a significant increase in LAB while the number of Enterobacteriaceae remained non-significant. Probiotic supplementation has been associated with improved movement of mucin and microbial populations in the chicken's small intestine, leading to enhanced intestinal function, health, and nutrient uptake (Fathima et al., 2022). The findings align with previous studies that demonstrate the positive effects of probiotics on broiler growth, nutrient digestibility, and changes in microflora composition (Fathima et al., 2022). Probiotics, such as *Lactobacillus* sp., have shown to improve growth, immune responses, and animal welfare by increasing gastrointestinal tract enzyme levels, promoting rapid digestion of nutrients, and maintaining microbial balance (Pertiwi and Mahendra, 2021).

The supplementation of probiotic *Lactobacillus* sp. in feed has been reported to significantly increase body weight in broiler chickens (Fesseha et al., 2021). However, the effectiveness of probiotics can be influenced by various factors, including host microbiota composition, probiotic dose and administration, host age and type, and the quality and type of probiotics used (Wang et al., 2021). Interestingly, the combination (parabiotic) of *Lactobacillus* sp., as observed in groups PI25 and PC10 (containing parabiotic from *Lactobacillus fermentum* and *Lactobacillus brevis*), exhibited immunomodulatory, antioxidant, and anti-inflammatory properties (Shin et al., 2023; Song et al., 2020). Furthermore, the parabiotic blend demonstrated inhibitory competition mechanisms against pathogen invasion, colonization, and adhesion, as well as preventing biofilm formation by pathogenic bacteria within host cells (Vinayamohan et al., 2024).

It is worth noting that poultry digestive tracts naturally harbor spoilage bacteria such as *Salmonella* sp. and *Staphylococcus* sp. (Salama and Chennaoui, 2024). *Lactobacillus fermentum*, which was used in this study, has been previously isolated from fermented plant material and evaluated for its hypocholesterolemic effects (Yang et al., 2021). Prior studies have indicated that probiotics incorporating *Lactobacillus fermentum* and *Saccharomyces cerevisiae* improved the intestinal balance of diverse microflora species in broiler chickens' rectum (Hati et al., 2023).

However, the effect of feeding freeze dried probiotic and parabiotic *Lactobacillus brevis* did not yield significant results concerning the fecal microflora of broiler chickens. This observation aligns with the findings of Son et al. (2023), who reported that *Lactobacillus brevis* strain B23 exhibited susceptibility to most antibiotics tested, with resistance observed in the cases of cefixime, nalidixic acid, vancomycin, and oxacillin.

## Conclusion

The supplementation of freeze-dried probiotic and parabiotic *Lactobacillus* sp., particularly *Lactobacillus brevis* C10, improved growth performance and increased lactic acid bacteria populations in broiler chickens, with more pronounced effects observed during the later stages of rearing. These findings confirm that both probiotic and parabiotic forms are capable of modulating gut microflora and supporting productive performance. Notably, parabiotic supplementation showed effects comparable to, and in some parameters exceeding, those of freeze-dried probiotics, suggesting its potential as a more stable alternative under high ambient temperature conditions. Therefore, parabiotic *Lactobacillus* sp. may represent a promising strategy to enhance broiler productivity and intestinal health, particularly in heat stress environments and commercial production systems. Further studies are warranted to elucidate the underlying mechanisms and to evaluate the consistency of these effects under varying management and environmental conditions.

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## Conflict of interest

There is not conflict of interest in this study

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