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STUDIES ON THE IMPACT OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS CASEI* CULTURED IN DIFFERENT NUTRIENT MEDIA ON THE GROWTH PERFORMANCE AND ON THE HELMINTHIASIS

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ABSTRACT

The overuse of antibiotics in broiler production raises public health concerns. This study evaluated the potential of Lactobacillus plantarum and Lactobacillus casei cultured in sweet potato and tempeh infusions as probiotic alternatives. A total of 150 broiler chicks were divided into three groups: control (P1), sweet potato-based probiotic (P2), and tempeh-based probiotic (P3). Probiotics were administered via drinking water for 35 days. Growth performance, including average daily gain (ADG) and final body weight (FBW) and helminth egg counts were assessed. Results showed that both probiotic treatments improved growth, with P3 showing significantly higher weight gain. Probiotic supplementation also reduced fecal egg counts of *Heterakis gallinarum*, *Ascaridia galli*, *Cysticola columbae*, and *Schistosoma mansoni*. The improvements are attributed to gut microbiota modulation and immunostimulatory effects of Lactobacillus spp. This study supports the use of sweet potato and tempeh infusions as cost-effective media for probiotics, offering a promising alternative to antibiotics in poultry farming.

Key words: Broiler, *Lactobacillus spp*., Poultry, Sweet potato, Tempeh.

Introduction

Over the past two decades, the use of antibiotics as growth promoters in livestock feed has been prohibited across European Union countries due to public health concerns associated with antimicrobial resistance (Pinho *et al.*, 2025). The European Commission officially enforced this ban through Regulation (EC) No. 1831/2003, which came into effect on January 1, 2006 (OECD, 2022). Since then, the sale of veterinary antimicrobials in 25 European countries has declined by approximately 53% between 2011 and 2022 (EMA, 2023), reflecting a significant transition toward more responsible antimicrobial stewardship. Globally, it is estimated that around 70–75% of all antimicrobials produced are used in food-producing animals, underscoring the substantial contribution of the livestock sector to overall antimicrobial consumption (FAO, 2017; OECD, 2022). These restrictions have consequently stimulated research into safer and more sustainable alternatives to maintain animal health and productivity.

The use of antibiotics in animal feed, particularly as growth promoters, may lead to the accumulation of residues in animal-derived products and accelerate the development of antibiotic-resistant bacteria, posing a major challenge to the poultry industry (Abou-Jaoudeh *et al.*, 2024). In parallel, the ongoing search for safe and effective anthelmintic treatments remains an urgent concern (Boros *et al.*, 2022). Among potential alternatives, probiotics have gained increasing attention due to their demonstrated anti-parasitic, antimicrobial, and immunomodulatory properties (Saracino *et al.*, 2021).

Within this context, *Lactobacillus* has emerged as the most widely used probiotic genus in poultry production, known to enhance growth performance, feed conversion efficiency, nutrient absorption, gut microbiota balance, intestinal health, and immune function in broilers (Pertiwi & Mahendra, 2021). However, the cultivation of these bacteria requires suitable media that can support their growth and ensure the successful isolation of potential probiotic strains (Bonnet *et al.*, 2020). An ideal culture medium should be cost-effective, practical, and capable of maintaining the viability and metabolic activity of *Lactobacillus* spp. (Aumiller *et al.*, 2023).

Formulating appropriate cultivation media remains challenging due to the fastidious nutritional requirements and strain variability of *Lactobacillus* spp. (Harina *et al.*, 2023). To promote optimal bacterial growth, culture media are typically enriched with amino acids, peptides, nucleic acid derivatives, fatty acid esters, minerals, vitamins, buffering agents, and carbohydrates (Rawat *et al.*, 2023). Common nitrogen sources such as soy peptones, tryptone, beef extract, or yeast extract are essential but often expensive, representing a major cost component in culture media production (Tao *et al.*, 2023; Yuniarti *et al.*, 2023). This economic constraint highlights the need for alternative, low-cost substrates capable of supporting bacterial proliferation without compromising growth efficiency.

Agricultural by-products and plant-derived materials have recently gained considerable attention as sustainable alternatives to conventional culture media components. Among these, sweet potatoes (*Ipomoea batatas*) represent a particularly promising substrate due to their high content of carbohydrates, amino acids, vitamins, and minerals, which can effectively support probiotic growth (Bernabeu *et al.*, 2024). Moreover, oligosaccharides derived from sweet potatoes function as prebiotics that stimulate the proliferation of *Lactobacillus* and enhance their survivability in the gastrointestinal environment (Nguyen *et al.*, 2021; Giang *et al.*, 2021).

In addition to sweet potatoes, tempeh—a traditional fermented soy-based product—also offers a nutrient-rich medium for probiotic cultivation. It provides abundant protein and bioactive compounds, including genistein, which contribute to the overall nutritional and functional quality of the culture substrate (Nurkolis *et al.*, 2024). Notably, co-fermentation of *Lactobacillus plantarum* with *Rhizopus oligosporus* during tempeh production has been reported to improve lipid metabolism and glucose regulation. Furthermore, *L. plantarum* produces β-glucosidase, an enzyme that hydrolyzes glucoside isoflavones into more bioavailable aglycones (Huang *et al.*, 2018). Collectively, the integration of natural substrates such as sweet potatoes and tempeh could enhance both the growth efficiency and functional potential of *Lactobacillus spp.* in probiotic applications for poultry production systems.

The functional benefits of these probiotics extend beyond bacterial cultivation. *Lactobacillus* species play a central role in maintaining intestinal microbiota homeostasis, which is critical for nutrient utilization, feed conversion efficiency, and immune defense against enteric pathogens (Naeem & Bourassa, 2025). Their metabolic activity facilitates nutrient absorption, thereby improving energy availability for growth and productivity (Abdel-Moneim *et al.*, 2020). Additionally, *Lactobacillus spp.* inhibit pathogen adhesion and colonization in the intestinal mucosa through competitive exclusion mechanisms, contributing to the prevention of enteric infections in broilers (Yaqoob *et al.*, 2022). These probiotics also produce bacteriocins and digestive enzymes that stabilize gut function, enhance nutrient digestibility, and modulate immune responses (Dempsey & Corr, 2022; Huang *et al.*, 2022; Huang *et al.*, 2023).

Considering the nutritional potential and prebiotic properties of sweet potato- and tempeh-based substrates, the present study aims to evaluate the effects of probiotic supplementation cultured in these alternative media on growth performance and helminth egg counts in broiler chickens.

Materials and Methods

The study was conducted in compliance with animal welfare and ethical regulations as stipulated in Indonesian Law No. 18/2009 on Animal Husbandry and Health and its amendment Law No. 41/2014. All experimental procedures followed national guidelines ensuring proper housing conditions, continuous access to feed and water, and appropriate environmental management to minimize stress and ensure animal welfare throughout the experimental period.

A completely randomized design (CRD) was employed in this study. A total of 150 day-old chicks (DOC) were randomly allocated into three treatment groups, each consisting of 50 birds: Control, P1: chickens received plain drinking water; P2: chickens received drinking water containing probiotics prepared in sweet potato infusion; P3: chickens received drinking water containing probiotics prepared in fermented soybean cake (tempeh) infusion.

All treatments were administered continuously for 35 days. During this period, the birds had no other water sources besides those containing the designated probiotic or control solutions.

Prior to administration, the probiotic preparation—containing *Lactobacillus casei* (1.62×10^6 CFU/mL) and *Lactobacillus plantarum* (0.25×10^6 CFU/mL) – was incubated in each infusion medium at room temperature (24–30 °C) for six days. The resulting probiotic solution was then provided to the broiler chickens via drinking water at a ratio of 15% probiotic infusion to 85% water.

The chicks used in this experiment were healthy, non-infected commercial broilers. No artificial infection was introduced; the presence of helminths was naturally occurring and monitored as part of the study. Body weight was recorded weekly to evaluate growth performance. In the 5-week, fecal samples were collected from each group to examine the presence of helminth eggs under a microscope at 100–400× magnification. All collected data were analyzed using one-way ANOVA with SPSS version 16. Differences between groups were considered statistically significant at $P < 0.05$.

Results

Supplementation with *Lactobacillus plantarum* and *Lactobacillus casei* in both sweet potato and fermented tempeh infusion media significantly increased the ADG and FBW of broilers compared to the control group ($P<0.05$). During the fourth week, the ADG of chickens in P3 (tempeh infusion) was 74.03 ± 17.27 g, which was significantly higher than both the control group P1 (39.69 ± 30.75 g) and the sweet potato infusion group P2 (39.51 ± 5.13 g). At the end of the 5-week experiment, the final body weight was highest in P3 (1409.0 ± 161.78 g), followed by P2 (1104.0 ± 83.23 g), and lowest in the control group P1 (1085.8 ± 77.15 g) (Table 1).

Table 1: Average daily gain and final body weight of broiler chicken drank with *Lactobacillus plantarum* and *Lactobacillus casei* in sweet potato and tempeh infusion water

Parameters	P1	P2	P3
Week 1	36.85±5.80	32.36±6.13	32.14±5.18
Week 2	23.02±12.95	28.71±12.05	27.00±8.78
Week 3	33.67±20.50	31.00±13.58	34.93±12.94
Week 4	39.69^{a}±30.75	39.51^{b}±5.13	74.03^{ab}±17.27
Final Body Weight	1085.8^{a} ±77.15	1104.0^{b}±83.23	1409.0^{ab}±161.78

Mean values bearing different superscript with in a row differ significantly (P<0.05)

Probiotic supplementation also affected the number of helminth eggs identified in the feces of broilers at the end of the 5-week experiment (Table 2). A significant decrease (*P*<0.05) was observed only in the number of *Heterakis gallinarum* eggs, which decreased from 620.0 ± 472.00 in P1 to 101.0 ± 1.00 in P2 and 101.0 ± 1.00 in P3. In contrast, the number of *Ascaridia galli* eggs increased from 101.50 ± 1.29 in P1 to 540.00 ± 173.20 in P2, while P3 showed 180.00 ± 138.56 eggs, and the differences were not consistent. Similarly, *Cysticola columbae* and *Schistosoma mansoni* eggs showed either minor increases or remained similar to the control (Table 2).

Table 2: Helminth eggs identification and total egg/gram feces of broiler chickens at the end of the 5-week experiment, drank with Lactobacillus plantarum and Lactobacillus casei in sweet potato and tempeh-infused water

Helmint Eggs	P1	P2	P3
Heterakis galinarum	620.0^{a} ± 472.00	101.0^{b} ± 1.00	101^{ab} ±1.00
Ascaridia galli	101.50^{a} ±1.29	540.00^{b} ±173.20	180.00^{ab} ±138.56
Cysticola columbae	160.25±119.83	180.33±138.27	101.00±1.00
Schistosoma mansoni	101.00^{a} ±0.81	180.00^{b} ±134.56	101.00^{ab} ±1.00

Mean values bearing different superscript with in a row differ significantly (P<0.05)

These results indicate that probiotic administration selectively suppressed *Heterakis gallinarum* infection at the end of the study, while showing limited or inconsistent effects on other helminth species. The data highlight that the effects of supplementation were species-specific, emphasizing the importance of monitoring individual helminth populations when evaluating probiotic efficacy.

Discussion

The results of this study demonstrated that supplementation with *Lactobacillus plantarum* and *Lactobacillus casei* cultured in sweet potato and tempeh infusion media significantly improved broiler growth performance. This was evident from the increase in average daily gain ADG and FBW compared to the control group. The highest improvement was observed in chickens receiving probiotics cultured in tempeh infusion, followed by those in sweet potato infusion. These findings indicate that the type of fermentation substrate plays an important role in determining the viability, stability, and metabolic activity of probiotics during fermentation and after entering the gastrointestinal tract (Rivas *et al.*, 2021).

Tempeh infusion medium is rich in peptides, free amino acids, and hydrolyzed isoflavones, which serve as nitrogen sources and bioactive compounds that support probiotic growth (Górska *et al.*, 2025). During fermentation, *Rhizopus oligosporus* converts soybean proteins into short peptides and amino acids that are readily utilized by *Lactobacillus* spp., thereby enhancing bacterial adaptability and metabolic activity in the intestine (Huang *et al.*, 2018). Furthermore, tempeh contains genistein and daidzein in their aglycone forms, which possess antioxidant and immunomodulatory properties, promoting beneficial microbial colonization and strengthening intestinal mucosal immunity (Ahmad *et al.*, 2015). The combination of these factors explains why the tempeh infusion group exhibited the best growth performance.

Meanwhile, the sweet potato infusion medium also exerted a positive effect on broiler performance, although not as pronounced as the tempeh medium. Sweet potatoes contain complex carbohydrates, resistant starch, and oligosaccharides such as raffinose and stachyose, which serve as fermentable energy sources and prebiotic compounds for *Lactobacillus* (Zaman & Sarbini, 2016; Nguyen *et al.*, 2021; Giang *et al.*, 2021; Tekin & Dincer, 2023). These oligosaccharides enhance probiotic viability by serving as key metabolic substrates for the production of lactic and acetic acids—two important metabolites that lower intestinal lumen pH and inhibit enteric pathogens such as *E. coli* and *Salmonella* (Tran *et al.*, 2018). In addition, the B-complex vitamins and minerals in sweet potatoes may support the enzymatic activity of *L. plantarum* and *L. casei*, enhancing their ability to synthesize hydrolytic enzymes such as proteases, amylases, and lipases, which contribute to improved nutrient digestibility (Islam, 2024). Thus, the sweet potato medium functions not only as a carrier but also as a functional substrate that reinforces probiotic metabolic activity and survival during gastrointestinal transit.

The significant improvement in growth performance observed in the fourth week suggests a period of microbial adaptation followed by stabilization of the intestinal ecosystem. *Lactobacillus* spp. produce organic acids and bacteriocins that reduce intestinal pH, creating an unfavorable environment for pathogens such as *Clostridium perfringens* and *Enterococcus faecalis*, while promoting colonization of beneficial bacteria (Monika *et al.*, 2024). This condition enhances villus height, reduces crypt depth, and increases the villus-to-crypt ratio, which translates into more efficient nutrient absorption. Additionally, *L. plantarum* and *L. casei* are known to produce bioactive metabolites such as exopolysaccharides (EPS) and short-chain fatty acids (SCFA), which serve as energy sources for enterocytes and help strengthen the intestinal mucosal barrier (Tao *et al.*, 2024).

From an immunological perspective, both *Lactobacillus* species can activate Toll-like receptor 2 (TLR2) and TLR4 on intestinal epithelial cells, leading to upregulation of mucin (MUC2) and tight junction proteins such as claudin, occludin, and ZO-1 (Al-Sadi *et al.*, 2021). This mechanism maintains epithelial barrier integrity against microbial and endotoxin invasion. Probiotics also enhance secretory IgA production and balance cytokine profiles by suppressing IL-6 and TNF-α while increasing IL-10 and TGF-β, resulting in an anti-inflammatory effect that supports immune homeostasis (Kumar *et al.*, 2025). These synergistic effects collectively improve feed efficiency and gut health, reflected in enhanced growth performance.

Regarding parasitic infection, the results showed that probiotic supplementation selectively reduced *Heterakis gallinarum* egg counts, whereas *Ascaridia galli*, *Cysticola columbae*, and *Schistosoma mansoni* remained unaffected. This species-specific response suggests that probiotic effects on helminth infection are dependent on parasite biology. The reduction in *Heterakis gallinarum* may be attributed to increased Th1-mediated immune responses via elevated IFN-γ, IL-2, and TNF-α production, which activate macrophages and suppress nematode development (Salas-Lais *et al.*, 2020; Tanaka *et al.*, 2020). Additionally, probiotics may modulate the microbial environment of the cecum – the primary site of *Heterakis gallinarum* development—through competition for space and nutrients, as well as secretion of antimicrobial metabolites that disrupt the parasite’s life cycle (Khasanah *et al.*, 2024).

Conversely, *Ascaridia galli* and *Schistosoma mansoni* may be less affected because they occupy distinct colonization sites (the anterior small intestine and mesenteric blood vessels, respectively), resulting in lower exposure to probiotic metabolites (Permin *et al.*, 2006). Furthermore, the five-week experimental duration may not have been sufficient to induce significant adaptive immune responses against parasites with longer life cycles. These findings are consistent with reports by Myhill *et al.* (2022) and Vinayamohan *et al.* (2024), which emphasize that probiotic efficacy against parasites depends on host species, infection site, and duration of exposure.

Overall, this study demonstrates that *L. plantarum* and *L. casei* cultured in tempeh- and sweet-potato-based media enhance broiler growth performance through mechanisms involving improved bacterial viability, modulation of gut microbiota, enhancement of mucosal morphology, and strengthening of mucosal immunity. The selective anthelmintic effect against *Heterakis gallinarum* indicates that probiotic–parasite interactions are species-specific and complex. Further studies are warranted to identify the key metabolites responsible for immunomodulatory activity, determine the optimal dosage, and explore the potential of natural fermentation media such as sweet potato and tempeh as functional carriers to enhance the biological efficacy of probiotics in tropical poultry production systems.

Conclusion

In conclusion, supplementation with the probiotics *Lactobacillus casei* (1.62×10^6 CFU/mL) and *Lactobacillus plantarum* (0.25×10^6 CFU/mL) in sweet potato and tempeh-infused water significantly improved broiler growth performance. Chickens receiving probiotics in tempeh infusion (P3) achieved the highest final body weight (1409 ± 161.78 g) at the end of the 5-week study, which was significantly higher than both the control group (P1: 1085.8 ± 77.15 g) and the sweet potato infusion group (P2: 1104.0 ± 83.23 g). Probiotic supplementation selectively reduced the number of *Heterakis gallinarum* eggs in feces at the end of the experiment (P3: 101 ± 1.00 eggs/g; P2: 101 ± 1.00 eggs/g) compared to the control (620 ± 472 eggs/g), whereas the numbers of *Ascaridia galli*, *Cysticola columbae*, and *Schistosoma mansoni* eggs either increased or remained unchanged. These results indicate that probiotic administration, particularly in tempeh-based media, enhances growth performance and provides species-specific protection limited to *Heterakis gallinarum* infection at the end of the study, highlighting the need for monitoring individual helminth populations when evaluating probiotic efficacy.

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