



# The Potency of Probiotics and Acidifiers on Body Weight, Feed Efficiency, Cholesterol, HDL, LDL in Meat of Broiler Chickens Infected with *Escherichia Coli*

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**Abstract** | This study aims to evaluate the effects of adding probiotics and acidifiers to feed as alternatives to antibiotic growth promoters (AGP) in broiler chickens infected with Avian Pathogenic *Escherichia coli* (APEC) concerning body weight, feed efficiency, meat quality, cholesterol, HDL, and LDL levels in chicken meat. Research method: preparation of experimental animals in vivo. The use of probiotics and acidifiers is used as a potential solution to improve intestinal health and optimize nutrient absorption. This study employed a Completely Randomized Design, consisting of six treatments, each with five replications, with each replication including two chickens, resulting in a total of sixty chickens used. The treatments are as follows: P0 = Broiler chickens without *E. coli* infection (Control -); P1 = Broiler chickens infected with *E. coli* (Control +); P2 = Broiler chickens infected with *E. coli* + AGP; P3 = Broiler chickens infected with *E. coli* + Probiotics; P4 = Broiler chickens infected with *E. coli* + acidifiers; P5 = Broiler chicken infected with *E. coli* + Probiotics + acidifiers. The observed variables included body weight, feed efficiency, cholesterol, HDL, and LDL levels in broiler chicken meat. The data obtained were then analyzed statistically using one-way Analysis of Variance (ANOVA). If the results were significantly different ( $p < 0.05$ ), Duncan's multiple range test was conducted. The results indicated that the addition of probiotics and acidifiers could increase body weight, enhance feed efficiency, and reduce cholesterol, LDL and improve HDL levels, all with significant effects ( $p < 0.05$ ).

**Keywords** | Probiotics, Acidifiers, Broiler, Feed additive, Zero hunger

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Poultry meat is a primary source of animal protein necessary to meet global protein needs. The primary challenge in increasing broiler chicken production is low feed efficiency and disruptions in digestive system metabolism caused by pathogenic microorganisms, such as *Escherichia coli*, which inhibit growth and compromise health. Colibacillosis is a disease caused by the pathogenic bacteria Avian Pathogenic *Escherichia coli* (APEC) and can lead to significant economic losses for the poultry industry globally (Ridhana *et al.*, 2021). In young broiler chickens, this infection is characterized by airsacculitis and polyserositis (including pericarditis, perihepatitis, and peritonitis), leading to high mortality in the first week, poor animal welfare, and economic losses for farmers (Kravik *et al.*, 2022; Kumari *et al.*, 2023).

In accordance with Law Number 18 of 2009 Juncto Number 41 of 2014 concerning Animal Husbandry and Animal Health, Article 22, Paragraph 4C, which prohibits the use of feed mixed with certain hormones and/or antibiotic feed additives, there is an opportunity to develop alternatives to Antibiotic Growth Promoters (AGP). One of the innovative feed additives to replace AGP is the use of probiotics, which can enhance immunity and livestock production. Another potential feed additive is acidifiers, which are beneficial for growth performance, meat quality, and the digestive health of broilers (Gao *et al.*, 2021). The use of these two feed additives could serve as an alternative to AGP for controlling pathogenic microbes and maintaining optimal livestock productivity (Sirisopapong *et al.*, 2023).

Probiotics are feed additives in the form of live and non-pathogenic microorganisms that are beneficial for improving the balance of microbes in the digestive tract, thereby increasing the efficiency and digestibility of feed. Several types of probiotics that have been used to enhance livestock growth performance include the probiotic *Lactobacillus casei* WB 315 (Yulianto *et al.*, 2020; Yulianto *et al.*, 2024), *Lactobacillus acidophilus*, *Lactococcus lactis*, *Bifidobacterium spp*, *Lactobacillus rhamnosus* (Lokapirnasari *et al.*, 2019; Lokapirnasari *et al.*, 2022; Lokapirnasari *et al.*, 2020; Lovela *et al.*, 2023; Kirana *et al.*, 2024) and *Pedio-coccus pentosaceus* ABY 118 to modulate the immune response (Yulianto *et al.*, 2021). The characteristics of *Lactobacillus sp.* include creamy white colonies, catalase-negative, Gram-positive, rod-shaped morphology (Sirisopapong *et al.*, 2023). *Lactobacillus acidophilus* affects cholesterol, HDL and LDL content (Lokapirnasari *et al.*, 2020). The use of *Lactobacillus sp* shows the effect of lowering cholesterol levels (Ali *et al.*, 2022). Even though cholesterol is essential for the formation of body tissue, hypercholesterolemia is one of the causes of coronary heart disease (CHD) (Sugiyama *et al.*, 2020). Several studies have demonstrated the

positive effects of using the probiotics *Lactobacillus casei* and *Lactobacillus rhamnosus* on total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels in broiler chickens (Andriani *et al.*, 2020).

Acidifiers (organic acids) are important feed additives for poultry as alternatives to AGP. Organic acids can serve as carbon and energy sources for microorganisms or as inhibitory agents, depending on the concentration of the acid, its ability to enter cells, and the capacity of the organism to metabolize the acid (Pearlin *et al.*, 2020). The antibacterial function of organic acids involves several mechanisms, including the lowering of pH, which creates an acidic environment that is incompatible with bacteria. In addition, organic acids can penetrate bacterial cell membranes and disrupt their integrity, leading to the loss of essential nutrients or the disruption of cellular metabolic processes (Scicutella *et al.*, 2021). Acidifiers have the potential to increase nutrient utilization, alter intestinal pH, and inhibit the growth of pathogenic microorganisms in the digestive system (Melaku *et al.*, 2021; Hamidifard *et al.*, 2023; Okey, 2023). The use of acidifiers in drinking water can suppress pathogenic bacteria and optimize the performance of broiler chickens (Zhang *et al.*, 2022). Benzoic acid as an acidifier can replace antibiotics to improve growth performance, enhance antioxidant properties, facilitate nutrient absorption, and help balance intestinal flora in broiler chickens (Wang *et al.*, 2020).

Therefore, the present study was conducted to investigate the effects of probiotics and acidifiers on body weight, feed efficiency, cholesterol, HDL, and LDL levels in broiler chickens infected with *Escherichia coli*.

## MATERIALS AND METHODS

### ETHICAL CLEARANCE

This study has received approval from the Universitas Airlangga University Ethics Study Team via a letter from the Chair of the Ethics Review Team, number 1.KEH.017.01.2024. The treatments in this study use probiotics and acidifiers as safe feed additives, which possess positive and beneficial characteristics, and do not cause pain to the test animals.

### STUDY LOCATION AND PERIOD

This study was conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, from March to July 2024.

### ANIMALS, EXPERIMENTAL DESIGN AND TREATMENT

The first stage involves the reculturing of probiotic isolates. Probiotic isolates are grown on MRSA and MRSB media under facultative aerobic conditions for 24 hours at 37°C. The probiotic isolates used were obtained from a collec-

tion of isolation and identification results based on the researcher's roadmap. The second stage involves the formulation of acidifiers, which is carried out independently. All ingredients consisting of *fumaric acid*, *lactic acid*, *DL malic acid*, *citric acid monohydrate* are weighed according to the formulation, mixed until homogeneous. The third stage: Preparation of Experimental Animals. Disinfection one week before the DOC arrives. On the first day, day-old chicks (DOC) are given drinking water mixed with 3% sugar. Feeding according to the manufacturer's recommendations for the Lohmann strain of chickens, and drinking is provided ad libitum for 5 weeks (35 days). After a one-week DOC adaptation period, 60 broiler chickens were divided into 6 treatments with 5 replications, each containing 2 broiler chickens. The treatments carried out were:

- P0: Broilers without APEC infection (control -).
- P1: Broilers infected with APEC (control +).
- P2: Broilers infected with APEC + standard drink + (feed + AGP (*Zinc Bacitracin* 0.1%).
- P3: Broilers infected with APEC + standard feed + (drink + probiotic 0.5%).
- P4: Broilers infected with APEC + standard drink + (feed + acidifiers 0.5%).
- P5: Broilers infected with APEC + (drink + probiotic 0.5%) + (feed + acidifiers 0.5%).

Chickens were infected with Avian Pathogenic *Escherichia coli* (APEC) orally with concentration  $10^8$  CFU/ml at 21 days of age. Provision of probiotics in the form of *Lactobacillus sp.* was provided at a dose of 0.5%, based on the optimal dosage identified in the study by Lokapirnasari *et al.* (2024). The administration of the probiotics was carried out by measuring 0.5% of the probiotics based on the amount of drinking water provided, then mixing it with the water before administration.

The AGP (zinc bacitracin) is administered at the time of feeding at a dose according to the recommended packaging (1 kg/ton of feed). The AGP is then mixed into the feed before being given.

The acidifier is administered to broiler chickens at a dose of 0.5%, which is the optimal dosage according to research conducted by Lokapirnasari *et al.* (2024). Specifically, the best dose of the acidifier (*fumaric acid*, *citric acid monohydrate*, *lactic acid*, *D-L malic acid*) for chickens is 0.5% of the feed. The acidifier is mixed into the broiler chicken feed and administered for 28 days (from 8 days to 35 days of age).

Probiotics, acidifiers, and AGP are administered daily starting from 8 to 35 days of age through drinking water and by mixing AGP into the feed. Chickens are infected orally with APEC at a dose of  $10^8$  CFU/ml at 21 days of age. Data collection is conducted at the end of each re-

search week, and samples of broiler chicken meat are taken at 35 days of age.

## DATA COLLECTION

The data on body weight and feed efficiency were collected every week. The analysis of cholesterol, HDL, and LDL was conducted at the end of the treatment. Filtrate extraction was performed for each treatment, and two meat samples were taken for each repetition. Cholesterol, HDL, and LDL levels were measured using a spectrophotometer (Li *et al.*, 2019; Jaya *et al.*, 2019).

## STATISTICAL ANALYSIS

Data were analyzed using *analysis of variance* (ANOVA). If a significant difference is found, *Duncan's Multiple Range Test* is conducted at a significance level of 0.05.

## RESULTS AND DISCUSSION

### BODY WEIGHT

Based on the statistical analysis results, there is a significant difference ( $p < 0.05$ ) between treatments affecting broiler body weight, as listed in the table below.

Based on the research results (Table 1), which were statistically tested using SPSS software, it was determined that the ANOVA results for the treatments given in the first and second weeks showed statistically non significant results ( $p > 0.05$ ). However, when tested against a negative control group (without *E. coli* infection; P0: 743.00 g/head), there was a significant difference in final body weight among groups P5 (802.00 g/head), P3 (803.40 g/head), and P4 (813.60 g/head). In the second week of treatment, a significant difference in final body weight was observed in groups P2 (1251.80 g/head), P5 (1275.00 g/head), P3 (1293.40 g/head), and P4 (1303.00 g/head). During the third week of treatment, the results were statistically significant ( $p < 0.05$ ). The treatment groups P1 (1671.00 g/head), P3 (1993.00 g/head), and P4 (2025.00 g/head) differed significantly from the negative control group (P0: 1798.20 g/head).

This research shows that the addition of an acidifier mixture has a positive impact on broiler performance. Acidifier can positively influence growth by reducing the acidity of feed and the intestine, eliminating harmful microbes sensitive to low pH, or selectively increasing *Lactobacillus*. Furthermore, the acid condition in digestive system can slow down stomach emptying, providing more time for nutrient digestion in the intestine. This result of research in accordance with Kumari *et al.* (2023) statement, that adding acidifier inside feed can increase the average daily gain (ADG) or increase body weight and reduce broiler feed conversion ratio (FCR). It is also stated that acidifier



supplementation at a dose of 0.1 g/kg can enhance the performance of broilers compared to the administration of antibiotics.

**Table 1:** Body Weight in broilers treated with probiotics and acidifiers.

| Duration of Treatment (Weeks) | Treatment Groups | Mean $\pm$ SD (g/head)             | P-Value |
|-------------------------------|------------------|------------------------------------|---------|
| 1 <sup>st</sup>               | P0               | 743.00 <sup>a</sup> $\pm$ 46.45    | 0.178   |
|                               | P1               | 772.60 <sup>ab</sup> $\pm$ 42.32   |         |
|                               | P2               | 791.00 <sup>ab</sup> $\pm$ 56.06   |         |
|                               | P3               | 803.40 <sup>ab</sup> $\pm$ 37.09   |         |
|                               | P4               | 813.60 <sup>b</sup> $\pm$ 50.82    |         |
| 2 <sup>nd</sup>               | P5               | 802.00 <sup>ab</sup> $\pm$ 30.94   | 0.007   |
|                               | P0               | 1195.80 <sup>a</sup> $\pm$ 102.71  |         |
|                               | P1               | 1167.60 <sup>a</sup> $\pm$ 32.50   |         |
|                               | P2               | 1251.80 <sup>ab</sup> $\pm$ 108.03 |         |
|                               | P3               | 1293.40 <sup>b</sup> $\pm$ 68.42   |         |
| 3 <sup>rd</sup>               | P4               | 1303.00 <sup>b</sup> $\pm$ 79.26   | 0.000   |
|                               | P5               | 1275.00 <sup>ab</sup> $\pm$ 98.30  |         |
|                               | P0               | 1798.20 <sup>b</sup> $\pm$ 83.81   |         |
|                               | P1               | 1671.00 <sup>a</sup> $\pm$ 69.68   |         |
|                               | P2               | 1723.00 <sup>ab</sup> $\pm$ 49.70  |         |
| Average                       | P3               | 1993.00 <sup>c</sup> $\pm$ 117.56  | 0.000   |
|                               | P4               | 2025.00 <sup>c</sup> $\pm$ 116.40  |         |
|                               | P5               | 1801.00 <sup>b</sup> $\pm$ 64.07   |         |
|                               | P0               | 1245.60 <sup>ab</sup> $\pm$ 59.16  |         |
|                               | P1               | 1203.80 <sup>a</sup> $\pm$ 28.91   |         |
|                               | P2               | 1255.20 <sup>ab</sup> $\pm$ 57.15  |         |
|                               | P3               | 1363.00 <sup>c</sup> $\pm$ 20.87   |         |
|                               | P4               | 1380.40 <sup>c</sup> $\pm$ 79.73   |         |
|                               | P5               | 1292.40 <sup>b</sup> $\pm$ 29.65   |         |

**Note:** (a,b,c) Different superscript letters within a column indicate statistically significant differences between treatment groups ( $p < 0.05$ ).

Group P3 also showed good growth, with a final body weight of approximately  $\pm 1993.00$  g/head, indicating that this treatment is effective, although not as effective as P4 ( $\pm 2025.00$  g/head). The control group (P0), without infection, showed a lower weight ( $\pm 1798.20$  g/head) compared to the group receiving the treatment. This indicates that the feed additive can have a positive impact on growth, even in the presence of infection. Acidifiers can improve the quality of villi intestine in chicken, particularly in terms of villi height. The increase in villi height in the small intestine of broiler chickens is closely related to enhanced digestive and absorption functions due to the expansion of the absorption area. This increase in villi height reflects the efficiency of the nutrient absorption system throughout the body, benefiting the host.

Therefore, the addition of acidifiers to broiler chicken feed can maximize nutrient absorption and increase body weight. Setyoko *et al.* (2020) stated that pH changes, supported by the addition of the papain enzyme as a proteolytic enzyme, can increase protein digestion and absorption because proteases work more effectively under low pH conditions. Optimal protein absorption will positively impact the improvement of broiler carcass weight.

Overall, the results in the broiler body weight table indicate that treatment with acidifiers and probiotics can enhance the growth performance of broiler chickens, even in the presence of *E. coli* infection. This emphasizes the importance of using appropriate feed additives to improve growth performance and chicken health while reducing the negative impact of infections. This research provides valuable insights for developing more effective feeding strategies in the chicken farming industry.

### FEED EFFICIENCY

Based on the statistical analysis results, there is a significant difference ( $p < 0.05$ ) between treatments regarding feed efficiency, as listed in the table below.

From the feed efficiency Table 2, it is clear that the group receiving the treatment combination of probiotics and acidifiers (P5) demonstrates the highest feed efficiency, with an average final feed efficiency of 64.21%. This indicates that this combination is very effective in maximizing feed use for weight gain. Group P4 also showed good feed efficiency, with an average final feed efficiency of 69.05%.

Group P0 has lower feed efficiency (65.17%), indicating that although it is not infected, its feed efficiency is not as good as that of the group receiving the treatment. This demonstrates that feed additive can have a positive impact on feed efficiency. From the analysis results, it appears that the group receiving AGP (P2) had moderate feed efficiency, with an average final feed efficiency of 58.17%. This indicates that although AGP can contribute to growth, its effectiveness in maximizing feed use is not comparable to the combination of probiotics and acidifiers. Group P1 shows lower efficiency (61.46%) compared to the groups that are not infected, confirming that chicken health significantly influences feed efficiency.

This research result shows that the use of probiotics and acidifiers can significantly increase feed efficiency in broilers. The use of probiotics as feed additives can improve growth performance in poultry. Probiotics and acidifiers can acidify the digestive tract, thereby lowering the pH, affecting the rate of digestion, and reducing the population of pathogenic bacteria, which positively impacts the health of the digestive tract and increases nutrient digestibility. Additional ingredients not only help increase weight gain

but also maximize feed utilization, which is an important factor in production efficiency in the farming industry. The use of probiotics is widespread among livestock breeders because they have various functions, including enhancing growth and feed efficiency and preventing intestinal inflammation and diarrhea. Using probiotics will be most effective if the pH of the chicken intestine is in accordance with the optimal pH for the growth of lactic acid bacteria. The others research showed that using probiotics (*L. acidophilus*, *Lactobacillus plantarum*, and *Bifidobacterium spp.*) could increase feed efficiency (Lokapirnasari *et al.*, 2024). So, the growth bacteria especially lactic acid bacteria can optimized with adding acidifier insiderations.

**Table 2: Feed Efficiency (FE) in broilers treated with probiotics and acidifiers.**

| Duration of Treatment (Weeks) | Treatment Groups | Mean $\pm$ SD (%)              | P-Value |
|-------------------------------|------------------|--------------------------------|---------|
| 1 <sup>st</sup>               | P0               | 66.16 <sup>a</sup> $\pm$ 3.31  | 0.000   |
|                               | P1               | 67.17 <sup>ab</sup> $\pm$ 3.45 |         |
|                               | P2               | 66.58 <sup>a</sup> $\pm$ 4.17  |         |
|                               | P3               | 71.85 <sup>c</sup> $\pm$ 2.12  |         |
|                               | P4               | 72.07 <sup>c</sup> $\pm$ 2.49  |         |
|                               | P5               | 71.04 <sup>bc</sup> $\pm$ 2.25 |         |
| 2 <sup>nd</sup>               | P0               | 64.94 <sup>a</sup> $\pm$ 4.76  | 0.007   |
|                               | P1               | 63.08 <sup>a</sup> $\pm$ 3.18  |         |
|                               | P2               | 63.34 <sup>a</sup> $\pm$ 4.93  |         |
|                               | P3               | 67.81 <sup>a</sup> $\pm$ 6.19  |         |
|                               | P4               | 68.80 <sup>a</sup> $\pm$ 2.73  |         |
|                               | P5               | 68.46 <sup>a</sup> $\pm$ 5.47  |         |
| 3 <sup>rd</sup>               | P0               | 65.17 <sup>bc</sup> $\pm$ 3.75 | 0.218   |
|                               | P1               | 61.46 <sup>ab</sup> $\pm$ 4.22 |         |
|                               | P2               | 58.17 <sup>a</sup> $\pm$ 2.53  |         |
|                               | P3               | 69.79 <sup>d</sup> $\pm$ 2.75  |         |
|                               | P4               | 69.06 <sup>cd</sup> $\pm$ 1.82 |         |
|                               | P5               | 64.21 <sup>b</sup> $\pm$ 2.83  |         |
| Average                       | P0               | 65.42 <sup>ab</sup> $\pm$ 2.50 | 0.000   |
|                               | P1               | 63.91 <sup>a</sup> $\pm$ 2.83  |         |
|                               | P2               | 62.70 <sup>a</sup> $\pm$ 3.41  |         |
|                               | P3               | 69.82 <sup>c</sup> $\pm$ 2.76  |         |
|                               | P4               | 69.98 <sup>c</sup> $\pm$ 1.99  |         |
|                               | P5               | 67.90 <sup>bc</sup> $\pm$ 1.70 |         |

**Note:** (a,b,c,d) Different superscript letters within a column indicate statistically significant differences between treatment groups ( $p < 0.05$ ).

## CONTENT CHOLESTEROL, HDL, LDL IN BROILER MEAT

Based on the statistical analysis results, there is a significant difference ( $p < 0.05$ ) between treatments regarding cholesterol content, as listed in the table below.

Table 3 shows that P2 has the highest cholesterol level (135.88 mg/100g), while P5 has the lowest cholesterol level (39.93 mg/100g). This difference is significant ( $p < 0.05$ ) between group P2 and the other groups, except for P1.

**Table 3: Average of cholesterol, HDL, LDL in broiler meat treated with probiotics and acidifiers.**

| Types of Results | Treatment Groups | Mean $\pm$ SD (mg/100g)          | P-Value |
|------------------|------------------|----------------------------------|---------|
| Cholesterol      | P0               | 75.41 <sup>ab</sup> $\pm$ 17.99  | 0.001   |
|                  | P1               | 100.11 <sup>bc</sup> $\pm$ 29.08 |         |
|                  | P2               | 135.88 <sup>c</sup> $\pm$ 48.27  |         |
|                  | P3               | 61.10 <sup>ab</sup> $\pm$ 14.75  |         |
|                  | P4               | 59.74 <sup>ab</sup> $\pm$ 14.75  |         |
|                  | P5               | 39.93 <sup>a</sup> $\pm$ 13.98   |         |
| HDL              | P0               | 60.68 <sup>b</sup> $\pm$ 4.95    | 0.000   |
|                  | P1               | 27.80 <sup>a</sup> $\pm$ 5.47    |         |
|                  | P2               | 15.52 <sup>a</sup> $\pm$ 6.03    |         |
|                  | P3               | 25.97 <sup>a</sup> $\pm$ 9.65    |         |
|                  | P4               | 30.45 <sup>a</sup> $\pm$ 10.79   |         |
|                  | P5               | 53.10 <sup>b</sup> $\pm$ 20.42   |         |
| LDL              | P0               | 55.03 <sup>a</sup> $\pm$ 7.29    | 0.044   |
|                  | P1               | 74.87 <sup>bc</sup> $\pm$ 1.07   |         |
|                  | P3               | 65.74 <sup>abc</sup> $\pm$ 1.75  |         |
|                  | P4               | 60.99 <sup>abc</sup> $\pm$ 17.71 |         |
|                  | P5               | 55.97 <sup>ab</sup> $\pm$ 8.79   |         |

**Note:** (a,b,c) Different superscript letters within a column indicate statistically significant differences between treatment groups ( $p < 0.05$ ).

Cholesterol content in broiler meat showed a significant difference between treatments ( $p < 0.05$ ). High cholesterol levels were observed in the P2 treatment (135.88 mg/100g), which were not significantly different from the P1 treatment (100.11 mg/100g). Low cholesterol levels were recorded in treatment P5 (39.93 mg/100g), followed by P4 (59.74 mg/100g) and P3 (61.10 mg/100g). Probiotics and acidifier treatments showed significant differences ( $p < 0.05$ ) between the treatments. High HDL levels were observed in the control treatment (P0: 60.68 mg/100g), which were not significantly different from treatment P5 (53.10 mg/100g). Probiotics and acidifier treatments showed significant differences ( $p < 0.05$ ) between the treatments. Low LDL levels were observed in the control treatment (P0: 55.03 mg/100g), which were not significantly different from treatments P5 (55.97 mg/100g), P4 (60.99 mg/100g), and P3 (65.74 mg/100g).

Cholesterol levels in broiler chicken meat can negatively impact human health, contributing to obesity, coronary heart disease, and hypertension (Utami *et al.*, 2018).

According to Andriani *et al.* (2020), probiotics with concentration  $10^8$  CFU/g through the feed and drinking water can change the ratio of LDL and HDL, so influence rate cholesterol compared to with administration of AGP. *Bacillus sp.* is capable of synthesizing the lipase enzyme, which breaks down fat into fatty acids and triglycerides, thereby decreasing cholesterol levels in the body. Blood and meat cholesterol levels are correlated, and lowering blood cholesterol levels can also reduce meat cholesterol levels. Feed manipulation, such as adding probiotic-rich fermented herbs as a feed supplement, may help lower cholesterol levels (Sumardi *et al.*, 2016).

According to research by Imran *et al.* (2021), the cholesterol levels in meat from broiler chickens given probiotics range from 66.63 to 79.47 mg per 100 grams. However, research by Candra and Putri (2020) found that adding turmeric powder to broiler chicks can reduce their cholesterol levels from 200 mg per 100 grams to 160 mg per 100 grams. This indicates that the addition of AGP in feed can increase cholesterol levels in meat, while the use of probiotics and acidifiers can lower cholesterol levels. The role of probiotic bacteria in reducing cholesterol levels in broiler chicken meat occurs through the absorption of cholesterol from intestinal micelles, which are then transported into the bacterial cell membrane through an assimilation process (Shehata *et al.*, 2019).

Krismiyo *et al.* (2023) stated that lactic acid can lower the pH of digestion and suppress pathogenic bacteria. The population of Lactic Acid Bacteria (LAB) is increasing, which can enhance the production of Bile Salt Hydrolases (BSH) enzymes that deconjugate bile salts, thereby improving the digestion and absorption of fat. The acidic atmosphere within the digestive tract limits the activity of the lipase enzyme, resulting in decreased fat digestion and body fat formation. This leads to meat with low cholesterol content.

## CONCLUSIONS AND RECOMMENDATIONS

The addition of 0.5% acidifiers in feed and 0.5% probiotics in the drinking water of broiler chickens demonstrates significant potency in enhancing growth performance, feed efficiency, and gut health, particularly in the context of pathogenic infections such as *Escherichia coli*. The study revealed that broilers receiving additives of probiotic and acidifier achieved higher body weights (e.g., P4:  $\pm 2025$  g/head) and improved feed efficiency (e.g., P5: 64.21%) compared to control groups, underscoring their effectiveness in promoting nutrient absorption and overall health. The results showed that the addition of probiotics and acidifiers could increase body weight, enhance feed efficiency, and

reduce cholesterol, LDL and improve HDL levels, with a significant effect ( $p < 0.05$ ). Future research should focus on addressing the efficacy of acidifiers and probiotics, including investigating their long-term effects on poultry health and meat quality, exploring their mechanisms of action, assessing the potential for resistance development in gut microbiota, and conducting comparative studies with other feed additives to evaluate their relative effectiveness compared to the others antibiotic growth promoters.

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## NOVELTY STATEMENT

This study was conducted to demonstrate that the administration of a combination of probiotics (*Lactobacillus sp.*) in drinking water and an acidifier in broiler feed infected with *Escherichia coli* resulted in the best performance, with the lowest cholesterol levels.

## AUTHOR'S CONTRIBUTIONS

Widya Paramita Lokapirnasari, Nanik Hidayatik, and Eka Pramyrtha Hestianah: Conceptualization and design of the study. Himatul Ilma Silfia, Muhammad Aviv Firdaus, and Ertika Fitri Lisnanti: Collected samples. Himatul Ilma Silfia, Muhammad Aviv Firdaus, and Ertika Fitri Lisnanti: Performed the laboratory procedures. A. Sherasiya, Andreas Berny Yulianto, Mirni Lamid, M. Anam Al-Arif: Analyzed and interpreted the data. Widya Paramita Lokapirnasari, Andreas Berny Yulianto: Writing-original draft. Nanik Hidayatik, Eka Pramyrtha Hestianah and Tabita Dameria Marbun: Writing review and editing. Widya Paramita Lokapirnasari, Himatul Ilma Silfia, Muhammad Aviv Firdaus and Zein Ahmad Baihaqi: Edited the manuscript. All authors have read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare that they have no competing financial interests or personal relationships that could be perceived to influence the work reported in this paper.

## REFERENCES

- Ali SM, Salem FE, Aboulwafa MM, Shawky RM (2022). Hypolipidemic activity of lactic acid bacteria: Adjunct



- therapy for potential probiotics. Plos one, 17(6): e0269953. <https://doi.org/10.1371/journal.pone.0269953>
- Andriani AD, Lokapirnasari WP, Karimah B, Hidanah S, Al-Arif MA, Soeharsono NH, Harijani N (2020). Effectiveness probiotics *Lactobacillus casei* and *Lactobacillus rhamnosus* as substitute for antibiotic growth promoter for total cholesterol, low density lipoprotein and high-density lipoprotein in broiler chickens. J. Med. Vet., 3(1): 114-122. <https://doi.org/10.20473/jmv.vol3.iss1.2020.114-122>
- Candra AA, Putri D (2020). Application of turmeric as antioxidant for broiler chickens. J. Phys. Con.. Ser., 1450(1): 1-6. <https://doi.org/10.1088/1742-6596/1450/1/012058>
- Gao CQ, Shi HQ, Xie WY, Zhao LH, Zhang JY, Ji C, Ma QG (2021). Dietary supplementation with acidifiers improves the growth performance, meat quality and intestinal health of broiler chickens. Anim. Nutr., 7(3): 762-769. <https://doi.org/10.1016/j.aninu.2021.01.005>
- Hamidifard M, Khalaji S, Hedayati M, Rajaei SH (2023). Use of condensed fermented corn extractives (liquid steep liquor) as a potential alternative for organic acids and probiotics in broiler ratio. Ital. J. Anim. Sci., 22(1): 418-429. <https://doi.org/10.1080/1828051X.2023.2206420>
- Imran Wajizah S, Samadi (2021). Influence of liquid probiotic inclusion as feed additives on lipid profiles and meat cholesterol content of commercial broiler chickens. IOP Conference series: Earth Environ. Sci., 667(1): 1-8. <https://doi.org/10.1088/1755-1315/667/1/012075>
- Jaya INS, Haryani NKD, Budi I (2019). Decline content cholesterol egg duck with giving onion fresh white with time different analysis. J. Vet. Sci., 61-68. <https://doi.org/10.22146/jsv.48517>
- Kirana ALP, Lovela AR, Lokapirnasari WP, Al-Arif MA, Hidanah S, Warsito SH, Ardianto (2024). Efficacy of probiotics on nutrient intake and egg weight in japanese quail (*Coturnix coturnix japonica*). J. Med. Vet., 7(2): 228-234. <https://doi.org/10.20473/jmv.vol7.iss2.2024.228-234>
- Kravik IH, Kaspersen H, Sjurseth SK, Jonsson M, David B, Aspholm M, Sekse C (2022). High sequence similarity between avian pathogenic *E. coli* isolates from individual birds and within broiler chicken flocks during colibacillosis outbreaks. Vet. Microbiol., 267: 109378. <https://doi.org/10.1016/j.vetmic.2022.109378>
- Krismiyananto L, Yunianto VD, Mangisah I, Crismawati R (2023). Feeding Rations Supplemented with Acidifiers, Inulin and Papain Enzymes on Fatty Meat and Carcass Weight of Grower-Phase Male Turkeys. J. Trop. Anim. Prod., 24(2): 71-83. <https://doi.org/10.21776/ub.jtapro.2023.024.02.2>
- Kumari M, Gupta RP, Bagri P, Singh R (2023). Immunopathological studies on *Escherichia coli* infected broiler chickens fed on Aloe vera leaf extract. Vet. Immunol. Immunopathol., 258: 110562. <https://doi.org/10.1016/j.vetimm.2023.110562>
- Li LH, Dutkiewicz EP, Huang YC, Zhou HB, Hsu CC (2019). Analytical methods for cholesterol quantification. J. Food Drug Anal., 27(2):375-386. <https://doi.org/10.1016/j.jfda.2018.09.001>
- Lokapirnasari WP, Pribadi TB, Al Arif A, Soeharsono S, Hidanah S, Harijani N, Najwan R, Huda K, Wardhani HC, Rahman NF, Yulianto AB (2019). Potency of probiotics *Bifidobacterium sp.* and *Lactobacillus casei* to improve growth performance and business analysis in organic laying hens. Vet. World, 12(6): 860. <https://doi.org/10.14202/vetworld.2019.860-867>
- Lokapirnasari WP, Sahidu AM, Maslachah L, Koestanti E (2020). Effect of *Lactobacillus casei* and *Lactobacillus acidophilus* in Laying Hens Challenged by *Escherichia coli* Infection. Sci. Malaysiana, 49(6): 1237-44. <https://doi.org/10.17576/jsm-2020-4906-03>
- Lokapirnasari WP, Agustono B, Al Arif MA, Maslachah L, Chandra EH, Yulianto AB (2022). Effect of probiotic and *Moringa oleifera* extract on performance, carcass yield, and mortality of Peking duck. Vet. World, 15(3): 694. <https://doi.org/10.14202/vetworld.2022.694-700>
- Lokapirnasari WP, Al-Arif MA, Maslachah L, Kirana ALP, Suryandari A, Yulianto AB, Sherasiya A (2023). The potency of *Lactobacillus acidophilus* and *L. lactis* probiotics and Guazuma ulmifolia Lam. extract as feed additives with different application times to improve nutrient intake and feed efficiency in Coturnix coturnix japonica females. J. Anim. Feed Sci., 32(1): 1-9. <https://doi.org/10.22358/jafs/156018/2022>
- Lokapirnasari WP, Al-Arif MA, Hidayatik N, Safranisa A, Arumdani DF, Zahirah AI, Yulianto AB, Lamid M, Marbun TD, Lisnanti EF, Baihaqi ZA (2024). Effect of probiotics and acidifiers on feed intake, egg mass, production performance, and egg yolk chemical composition in late-laying quails. Vet. World, 17(2): 462. <https://pmc.ncbi.nlm.nih.gov/articles/PMC11000483/>
- Lovela AR, Lokapirnasari WP, Warsito SH, Prasinta R, Hapsari T, Andriani A (2023). The Quality of Japanese Quail Eggs After Administration of Bifidobacterium sp. and Guazuma ulmifolia Leaf Extract. J. Med. Vet., 6(1): 132-136. <https://doi.org/10.20473/jmv.vol6.iss1.2023.132-136>
- Melaku M, ZhongR, Han H, Wan F, Yi B, Zhang H (2021). Butyric and citric acids and their salts in poultry nutrition: Effects on gut health and intestinal microbiota. Int. J. Mol. Sci., 22(19):10392. <https://doi.org/10.3390/ijms221910392>
- Okey SN (2023). Alternative Feed Additives to Antibiotics in Improving Health and Performance in Poultry and for the Prevention of Antimicrobials: A Review. Niger. J. Anim. Sci. Technol. (NJAST), 6(1):65-76. <https://njast.com.ng/index.php/home/article/view/243/226>
- Pearlin BV, Muthuvel S, Govidasamy P, Villavan M, Alagawany M, Ragab Farag M, Dhama K, Gopi M (2020). Role of acidifiers in livestock nutrition and health: A review. J. Anim. Physiol. Anim. Nutr., 104(2): 558-569. <https://doi.org/10.1111/jpn.13282>
- Ridhana F, Rahmawati, Makmur A (2021). The effect of feeding acidifier and probiotics with different levels in the diet growth on broiler performance. 12 (2): 28-35. <https://doi.org/10.30736/jt.v12i2.91>
- Scicutella F, Mannelli F, Daghighio M, Viti C, Buccioni A (2021). Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: a review. Antibiotics, 10(8): 1010. <https://doi.org/10.3390/antibiotics10081010>
- Setyoko H, Sukanto B, Wahyono F, Krismiyananto L (2020). Crude fat digestibility and weight carcass broiler chickens consequences addition extract fruit noni (*Morinda citrifolia* L.) and *Lactobacillus acidophilus*. E-Proceedings of the National Science Seminar Farm Applied. Jember, 19-22 September 2020:134-142. <https://doi.org/10.25047/proc.anim.sci.2020.19>
- Shehata MG, El-Sahn MA, El Sohaimy SA, Youssef MM (2019). role and mechanisms lowering cholesterol by dietary of probiotics and prebiotics. J. Appl. Sci., 19(8): 737-746. <https://scialert.net/fulltext/?doi=jas.2019.737.746>

- Sirisopapong M, Shimosato T, Okrathok S, Khempaka S (2023). Assessment of lactic acid bacteria isolated from the chicken digestive tract for potential use as poultry probiotics. *Anim. Biosci.*, 36(8): 1209. <https://doi.org/10.5713/ab.22.0455>
- Sugiyama D, Turin TC, Yeasmin F, Rumana N, Watanabe M, Higashiyama A, Takegami M, Kokubo Y, Okamura T, Miyamoto, Y (2020). Hypercholesterolemia and lifetime risk of coronary heart disease in the general Japanese population: Results from the Suita cohort study. *J. Atheroscler. Thromb.*, 27(1):60-70. <https://doi.org/10.5551/jat.49098>
- Sumardi S, Susanto GN, Kurtini T, Hartono M, Puspitaningsih. (2016). Effect of probiotik on blood cholesterol in laying hens. *Indones. J. Vet. Sci.*, 10(2): 128-131. <https://doi.org/10.21157/j.ked.hewan.v10i2.5042>
- Utami MMD, Pantaya D, Agus A (2018). Addition of garlic extract in ratio to reduce cholesterol level of broiler. *J. Phys. Conf. Ser.*, 953(1): 1-6. <https://doi.org/10.1088/1742-6596/953/1/012124>
- Wang Y, Wang Y, Lin X, Gou Z, Fan Q, Ye J, Jiang S (2020). Potential effects of acidifier and amylase as substitutes for antibiotics on the growth performance, nutrient digestion and gut microbiota in yellow-feathered broilers. *Animals*, 10(10):1858. <https://doi.org/10.3390/ani10101858>
- Yulianto AB, Lokapirnasari WP, Najwan R, Wardhani HCP, Rahman NFN, Huda K, Ulfah N (2020). Influence of *Lactobacillus casei* WB 315 and crude fish oil (CFO) on growth performance, EPA, DHA, HDL, LDL, cholesterol of meat broiler chickens. *Iran. J. Microbiol.*, 12(2): 148. <https://pubmed.ncbi.nlm.nih.gov/32494349/>
- Yulianto AB, Suwanti LT, Widiyatno TV, Suwarno S, Yunus M, Tyasningsih W, Hidanah S, Sjoftan O, Lokapirnasari WP (2021). Probiotic *Pediococcus pentosaceus* aby 118 to modulation of chifn - $\gamma$  and chil-10 in broilers infected by eimeria tenella oocyst. *Vet. Med. Int.*, 2021: 1-6. <https://doi.org/10.1155/2021/1473208>
- Yulianto AB, Lamid M, Lokapirnasari WP, Al-Arif MA, Sherasiya A, Marbun TD, Khairullah AR, Kurniawan SC, Hasib A, Silaen OSM, Rehman S (2024). Hematological and performance variables of male broiler chickens fed with moringa oleifera extract and probiotic in drinking water. *Trop. Anim. Sci. J.*, 47(2):215-224. <https://doi.org/10.5398/tasj.2024.47.2.215>
- Zhang H, Guo Y, Wang Z, Wang Y, Chen B, Du P, Zhang X, Huang Y, Li P, Michiels J, Chen` W (2022). Acidification of drinking water improves tibia mass of broilers through the alterations of intestinal barrier and microbiota. *Anim. Biosci.*, 35(6):902. <https://hdl.handle.net/1854/LU-8746543>