Hasanuddin J. Anim. Sci. Vol. 7, No. 1: 65-76
pISSN 2621-9182 eISSN 2621-9190
DOI: 10.20956/hajas.v7i1.42991



# Prebiotic, Probiotic, and Synbiotic Effect on Performance and Small Intestinal Morphology of Broiler Chicken

## A.F. Assaf[1*], D.P. Rahardja[2], and S. Purwanti[3]

1- Graduate student of Animal Science and Technology, Faculty of Animal Science, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
2- Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
3- Department of Animal Nutrition, Faculty of Animal Science, Hasanuddin University, Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
*Corresponding author- E-mail: ahmadfuadassaf06@gmail.com; djonipra@gmail.com

***How to Cite: Assaf A.F., D.P. Rahardja, and S. Purwanti. 2025. Prebiotic, Probiotic, and Synbiotic Effects on Performance and Small Intestinal Morphology of Broiler Chicken. Hasanuddin Journal of Animal Science, 7(1): 65–76 https://Doi. 10.20956/hajas.v7i1.42991.***

ARTICLE INFO

Article history:
Submission: January 21, 2025
Accepted: May 30, 2025
Published: October 20, 2025

ABSTRACT

The study was conducted to investigate the effect of prebiotic, probiotic, and synbiotic supplementation through drinking water on broiler performance and small intestine morphology. A total of 100 day-old broiler chicks (Ross) were randomly allocated into 4 treatments with 5 replicates, and there were 5 broilers in each experimental unit for 35 days. The drinking water treatments were P0: Water (Control); P1: Water + Prebiotic (5 g/L); P2: Water + Probiotic (3 g/L); P3: Water + Synbiotic (Probiotic 3 g/L + prebiotic 5 g/L). Commercial feed consisting of the three rearing phases was used during the study, and drinking water was provided *ad libitum*. Parameters measured were water and feed consumption, body weight gain, feed conversion, performance index, small intestine morphometry, which is measured by the length/weight ratio, and Histo-morphometry of the small intestine of individual segments, duodenum, jejunum, and ileum at the end of the experiment on 3 chicken samples (lightest, medium, heavy) from each treatment. The results showed that prebiotic, probiotic, and synbiotic supplementation through drinking water significantly ($P<0.05$) affected drinking water and feed consumption, body weight gain, feed conversion, length/weight ratio, and histo-morphology of the small intestine. Overall, it can be concluded that synbiotic supplementation showed a synergistic effect between prebiotics and probiotics, which was better than prebiotic and probiotic supplementation separately, on performance, small intestine ratio, and small intestine histomorphology. Synbiotics also significantly showed a trend towards feed use efficiency, as seen in feed conversion.

**Keywords:** Broiler Chicken, Natural Growth Promotor, Performance Index, Intestinal Gut Ratio and Intestinal Histomorphometry

## INTROODUCTION

A short-term broiler chicken maintenance period (28-35 days), which could reach 2kg bodyweight, is characterized by an intent to reduce disease risk in the gut, leading to the use of *antibiotics* as a protective measure. Antibiotic Growth Promoters (AGPs) could increase performance and animal health by preventing infection and digestive disease, reducing morbidity, mortality, and improving feed conversion ratio [1]. Thus, *the enhancement of antibiotic* usage has led to antimicrobial resistance and antibiotic residue in animal food products [2], as well as environmental contamination, which threatens animals and consumers [3]. Probiotic supplementation as a living microbe through water drinking and diet has shown an increased growth performance [4]. Previous studies have shown that probiotics could enhance nutrient absorption [5], improve gut health, and boost the growth performance of broilers [6], as well as increase villus height and crypt depth [7]. Nevertheless, prebiotics are needed for better probiotic bacteria viability in the gut because they cannot be digested by the digestive system and are instead digested by probiotic bacteria.

Prebiotics are defined as undigested carbohydrates and have a beneficial influence on the host. Prebiotics have been confirmed to have their potential on stimulating good bacteria growth in the digestive systems, hold hydrolitic activity in the top part of the digestive systems, and increase *short-chain fatty acid* production. Prebiotic supplementation through water drinking has been shown to improve *Lactic Acid Bacteria* and other beneficial bacteria in digestive systems, as well as increase the growth performance of broiler chickens [8] and improve gut morphology [9]. Synbiotics are defined as a probiotic and prebiotic combination that provides a synergic effect between both, which selectively increases viability, probiotic competition, and metabolic activity in the gut that could increase villus height, width, surface area, and crypt depth, so nutrient absorption could be more optimal, which will provide a positive effect on broilers' performance [10].

Based on several studies conducted, one parameter that could be used to evaluate chicken growth, whether it's good or poor, is to assess its quality or internal organ size, such as gut histology structure [11]. Digestive ability and absorption are influenced by villus height, surface area, and crypt depth [12]. Thus, this research was designed to investigate the effects of a combination of probiotics and prebiotics through water drinking supplementation on the performance and small intestinal morphology of broiler chickens.

## MATERIALS AND METHODS

The tools used in this research included an analytical scale, feeder and gallons, lamp, heater, thermometer, disinfectant solution, basins, shovels, knives, scissors, gloves, trays, prep glass, suction paper, microscopes, and microtomes. The materials used in this study were broiler chicken, commercial probiotic and prebiotic, commercial feed of starter, grower, and finisher phases, water, aquades solution, formalin, alcohol, xylol, parafin, and haematoxylin-eosin. The research was conducted from January to February 2024. The stages of this research involve supplementing prebiotics, probiotics, and synbiotics through water drinking to assess the performance and small intestinal morphology of broiler chickens at the Animal Production

Laboratory and the Animal Physiology Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar.

### Research Procedure

In the research on prebiotic, probiotic, and synbiotic supplementation, a total of 100 one-day-old Ross broiler chickens were used, arranged in a completely randomized design with 4 treatment groups, each consisting of 5 replicates of 5 chickens. Supplementation was through drinking water during the experiment (28 d): P0=control, water without any supplementation, P1= prebiotic/inulin (BENEO$^{tm}$ IPS Orafi Belgium) (5 g/L); P2= commercial probiotic (3 g/L), and P3= synbiotic (mix of 5 g prebiotic + 3 g probiotic /L). A commercial probiotic used contains bacteria *Bacillus subtilis* (>1 x $10^8$ CFU/g), *Bifidobacterium bifidum* (>1 x $10^{8)}$ CFU/g, *Bifidobacterium longum* (>1 x $10^8$ CFU/g), and *Lactobacillus bulgaricus* (>1 x $10^8$ CFU/g Use according to the dosage of prebiotic and probiotic. Each treatment unit of 5 chickens was placed in a square bamboo cage of (150 x 150 x 100) cm$^3$ with a floor of rice husk.

The chickens had free access to feed and water and were monitored daily. Data was accumulated over the course of the experiment, along with weekly body weight monitoring. There was one brand of commercial feed provided, which consisted of three rearing phases, namely SB10-crumble (1-7 d), SB11-crumble (8-21 d), and SB12-pellet (22-28 d - harvest) (Table 1)

Table 1. Nutrient specifications of different phases of the feed

| Nutrient | | Feed | | |
|---|---|---|---|---|
| | | SB10 | SB11 | SB12 |
| Water content | Maximum | 14 | 14 | 14 |
| Crude protein | Minimum | 22 | 20 | 19 |
| Crude fat | Minimum | 5 | 5 | 5 |
| Crude fiber | Maximum | 4 | 4 | 6 |
| Ash | Maximum | 8 | 8 | 8 |
| Calsium | - | 0.80 - 1.10 | 0.80 - 1.10 | 0.80 - 1.10 |
| Phosporus | Minimum | 0.50 | 0.50 | 0.45 |
| Amino acid % | | | | |
| Lysine | Minimum | 1.30 | 1.20 | 1.05 |
| Methionine | Minimum | 0.50 | 0.45 | 0.40 |
| Methionine + Cystin | Minimum | 0.90 | 0.80 | 0.75 |
| Threonine | Minimum | 0.80 | 0.75 | 0.65 |
| Tryptophan | Minimum | 0.20 | 0.19 | 0.18 |

Source: Brochure of produced animal feed PT. Charoen Pokphand Indonesia.

### Gross-Morphology Indices of Small Intestine

The three chickens from each treatment group were used in samples for the examination of gross-morphometric indices of the small intestine. All chicken samples were slaughtered at d 35. Gross morphometric indices of the small intestine are measures of the length/weight ratio of individual segments, the duodenum, jejunum, and ileum. This reflects the different functions of individual segments in absorption processes and digestion. The small intestine was aseptically emptied by gently flushing twice with isotonic saline solution to remove the luminal digest.

### Histomorphology of Small Intestine

Approximately 2-3 cm of the middle parts of the duodenum, jejunum, and ileum were cut as intestinal samples for histo-morphometry examination. The samples of each segment were fixed in a 10% formalin buffered solution and soaked for 24 h. Following fixation, the samples were processed through a series of alcohols with increasing concentrations (70, 80, 90, and 95%). The samples were transferred one by one to each alcohol concentration and allowed to soak for about 10 s. Then, the sample is inserted into xylol and finally immersed in paraffin wax. After paraffin moulding, 4 slices of 4 µm thick were prepared from each sample using a microtome, and fixed on a glass slide, then stained with hematoxylin-eosin. The histological preparations on the glass slide were observed and measured using a computer microscope, Zeiss Primo Star, equipped with an OptiLab Projector (camera). A histological picture appears on the monitor screen, Optilab viewer 2.2. The histo-morphometry measures comprised villi height (VH), apical width (AW), basal width (BW), and crypt depth (CD) [13].

### Data analysis

Quantitative data were analyzed using the statistical package SYSTAT version 13.2 [14], based on a one-way Analysis of Variance in a randomized complete block design. Qualitative data were analysed using interpretation from the literature study and compared with the results of another research.

## RESULTS AND DISCUSSIONS

### Performance

The effect of prebiotic, probiotic, and synbiotic supplementation through drinking water on the performance is presented in Table 2. The supplementation of prebiotics, probiotics, and synbiotics showed significant effects ($P<0.05$) on broiler performance.

Table 2. Supplementation of probiotic, prebiotic, and synbiotic effects through drinking water on the performance of broiler for 35 days

| Parameters | P0 | P1 | P2 | P3 | P-Value |
|---|---|---|---|---|---|
| Water Intake (ml/bird) | 6172.55±21.44[a] | 6271.60±22.52[c] | 6306.10±25.47[d] | 6262.34±29.96[b] | ** |
| Feed Intake (g/bird) | 3203.98±20.33[a] | 3203.27±23.09[a] | 3253.01±28.04[b] | 3257.44±23.07[b] | ** |
| Weight Gain (g/bird) | 2033.87±15.62[a] | 2034.60±12.12[a] | 2078.53±11.94[b] | 2084.38±13.79[b] | ** |
| Feed Conversion Ratio | 1.583±0.01[b] | 1.574±01[a] | 1.565±0.01[a] | 1.562±0.01[a] | ** |
| Performance Index | 370.09±2.17[a] | 377.07±1.67[b] | 387.16±2.73[c] | 388.03±1.21[c] | ** |

P0: control; P1: prebiotic; P2: probiotic; P3: synbiotic. Mean Values in the same row followed by different letters indicate a significant difference at the level P≤0.05.

The highest water intake was observed in P2 (probiotic) treatment at 6306.10±25.47 ml/bird. Birds supplemented with synbiotic (P3) demonstrated the highest feed intake (3257.44±23.07 g/bird) and weight gain (2084.38±13.79 g/bird). The best Feed Conversion Ratio (FCR) was achieved by P3 with a value of 1.562±0.01, indicating better feed efficiency compared to other treatments. Additionally, P3 reached the highest Performance Index (388.03±1.21), suggesting that synbiotic supplementation provided the best overall performance compared to other treatments.

There has been a concerted effort to develop potential alternatives to in-feed antibiotic growth promoters as the limitations on their use in animal production have intensified [15]. Numerous studies have demonstrated that synbiotics, composed of probiotics and prebiotics that act synergistically, can be used as effective alternatives to in-feed antibiotics [16].

The effect of synbiotic supplementation is primarily attributed to the beneficial microorganisms and substrates that facilitate the growth of small intestinal microorganisms, which serve as potential modulators of gut health and growth performance. Tang et al. [17] reported that the combination of probiotics and prebiotics creates a synergistic effect that enhances beneficial bacterial colonization in the digestive tract, contributing to better FCR in the P3 group. Incorporating prebiotics and probiotics as symbiotics in drinking water, as demonstrated in the current study, may stimulate the production of digestive enzymes such as amylase, lipase, and protease, potentially improving the digestion and absorption of nutrients [18].

The improvement in performance parameters can be explained through multiple pathways. Liu et al. [19] reported that synbiotics effectively modulate gut microbiota composition by increasing *Lactobacillus* and *Bifidobacterium* populations. Moreover, beneficial bacteria such as *Lactobacillus spp, Bacillus spp, and Bifidobacterium spp*. are known to produce digestive enzymes which could help to enhance digestion and improve feed conversion in host animals [20]. In the current study, supplementation of prebiotic (inulin), probiotics (containing *Bacillus subtilis, Bifidobacterium longum, Bifidobacterium bifidum,* and *Lactobacillus bulgaricus),* or in a combination as synbiotic may result in modulation of a beneficial microbiota, generally to increase their population, and to limit growth and attachment sites of harmful or pathogen microorganism on the intestinal surface [21]. Previous studies by Biswas et al. [22] support these findings, demonstrating that prebiotics improve microbial balance in the gastrointestinal tract through bacterial antagonisms, immune organ stimulation, and competitive exclusion. The non-digestible oligosaccharides in prebiotics help control bacterial activity and microbial composition, maintaining beneficial bacteria that suppress pathogen growth through various regulatory mechanisms. Furthermore, the synbiotic combination improves the survival rate of probiotics during their passage through the digestive tract, enhancing and stabilizing the probiotic effects.

The current study's results align with recent research trends highlighting the superiority of synbiotics compared to individual prebiotic or probiotic supplementation. The observed performance improvements can be attributed to the synergistic effects of combining probiotics and prebiotics, which optimize gut health and nutrient utilization efficiency.

### Gross Morphometry

Gross morphometry indices of the small intestine presented as Length/Weight ratios of individual segments indicated that there was a significant effect of prebiotic, probiotic, or synbiotic supplementation through drinking water (Table 3).

Table 3. Supplementation of prebiotic, probiotic, and synbiotic through drinking water on the length-weight ratio (LWR) of the broiler each segment small intestine for 35 days

| Parameters | Treatment | | | | P-value |
|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | |
| Intestinal Segment (cm/g) | | | | | |
| Duodenum | 4.39±0.20$^{a}$ | 5.66±0.26$^{bc}$ | 5.48±0.22$^{b}$ | 6.00±0.51$^{c}$ | * |
| Jejunum | 5.18±0.08$^{a}$ | 6.13±1.12$^{b}$ | 6.52±0.07$^{c}$ | 7.09±0.39$^{d}$ | * |
| Ileum | 4.96±0.05$^{a}$ | 5.45±0.22$^{b}$ | 5.46±0.09$^{b}$ | 6.48±0.15$^{c}$ | * |

P0: Control; P1: prebiotic; P2: probiotic; P3: synbiotic. Mean Values in the same row followed by different letters indicate a significant difference at the level (P≤0.05)

Statistical analysis presented in Table 3 showed that supplementation of prebiotic, probiotic, and synbiotic through drinking water significantly affected (P<0.05) the length-weight ratio of individual segments of the small intestine in broilers. The synbiotic treatment (P3) demonstrated the highest length-weight ratio across all intestinal segments, with duodenum (6.00±0.51 cm/g), jejunum (7.09±0.39 cm/g), and ileum (6.48±0.15 cm/g), showing significant differences (P<0.05) compared to other treatments. Both prebiotic (P1) and probiotic (P2) treatments showed intermediate values that were significantly higher than the control (P0) but lower than the synbiotic treatment (P3).

There is a dearth of reports linking the effects of nutrient supplementation and especially that of supplementation of prebiotic, probiotic, or symbiotic, on the length and/or weight or the length/weight ratio of the whole or individual segment of the small intestine. Alteration in the length/weight ratio of individual segments indicate the opportunity for longer or shorter time of digestion and absorption processes, while alteration in villi height (VH) or crypt depth (CD) or villus surface area (VSA) or VH/CD ratio [23] are an indication of mucosal thickness alteration which is associated with enzymatic processes and absorption surface area [24]**.** A comparison study between Lohmann dual purpose (LD) and Ross 308 Broiler line [25] indicated that with the same body weight (but different ages achieved), LD had a significantly heavier gizzard, shorter intestine, longer jejunal villi, and thicker ileal tunica muscularis than those found in Ross 308. The increased length-weight ratios across all intestinal segments suggest improved intestinal mass and absorption surface area.

The values are presented as the length-to-weight ratio of each segment. It is an approach to indicate the specific structure and function of each intestinal segment, which varies among the three segments along the small intestine [26]. A higher value of the ratio means that one gram of the segment has a longer and thinner segment. An increase in the length/weight ratio of intestinal gross morphometry and intestinal villus of histo-morphometry may enhance the digestive and absorptive functions of the intestine, resulting from an increased absorptive area and increased activity among intestinal brush border enzymes [27].

### Histo-Morphometry of Small Intestine

Histo-Morphology of the Small intestine of individual segments indicated that there was a significant effect of prebiotic, probiotic, or synbiotic supplementation on the drinking water (Table 4). Supplementation of prebiotic, probiotic, and synbiotic through drinking water showed significant effects (P<0.05) on small intestine morphology in broilers.

Table 4. Supplementation of prebiotics, probiotics, and synbiotics through drinking water on the histo-morphometry of broiler each segment small intestine for 35 days

| Parameters | Treatment | | | | P-value |
|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | |
| Duodenum | | | | | |
| Crypth Depth | 144.03±24.91[a] | 214.94±18.42[b] | 229.82±34.55[b] | 240.04±6.60[b] | * |
| Villus Height | 458.17±18.89[a] | 753.69±39.99[b] | 866.92±23.59[c] | 1,420.31±72.34[d] | * |
| VH/ CD Ratio | 3.24±0.50[a] | 3.53±0.43[a] | 3.85±0.66[a] | 5.92±1.01[b] | * |
| Vili Area ($mm^2$) | 584.10±21.63[a] | 930.70±42.77[b] | 1,025.80±42.14[c] | 1,567.81±58.60[d] | |
| Jejenum | | | | | |
| Crypth Depth | 204.52±2.01[a] | 263.41±1.97[b] | 266.17±14.62[b] | 283.40±4.81[c] | * |
| Villus Height | 558.17±18.89[a] | 851.16±44.49[b] | 916.92±54.79[c] | 1,216.43±23.52[d] | * |
| VH/ CD Ratio | 2.73±0.10[a] | 3.23±0.18[b] | 3.44±0.05[c] | 4.29±0.74[d] | * |
| Vili Area ($mm^2$) | 738.16±16.71[a] | 994.44±40.36[b] | 1,096.91±54.69[c] | 1,372.71±41.97[d] | |
| Ileum | | | | | |
| cripta Depth | 136.23±8.16[a] | 161.37±2.11[b] | 168.19±1.98[b] | 241.73±8.85[c] | * |
| Vilius Height | 638.40±31.28[a] | 853.69±39.99[b] | 891.92±63.59[b] | 1,341.43±77.59[c] | * |
| VH/ CD Ratio | 4.69±0.23[a] | 5.29±0.18[b] | 5.31±0.44[b] | 5.55±0.34[b] | * |
| Vili Area ($mm^2$) | 748.18±32.28[a] | 967.73±41.56[b] | 1,056.91±64.38[c] | 1,526.42±75.79[d] | |

P0: Control; P1: prebiotic; P2: probiotic; P3: synbiotic. Mean Values Within the same row, followed by the different letters below, are significantly different (P<0.05)

In the duodenum, synbiotic supplementation (P3) resulted in the highest crypt depth (240.04±6.60), villus height (1,420.31±72.34), VH/CD ratio (5.92±1.01), and vili area (1,567.81±58.60) compared to other treatments. Similarly, in the jejunum and ileum. The intestinal microbiota plays an important role in maintaining a stable gut environment through its impact on numerous physiological processes, including intestinal development and function, micronutrient synthesis, and immune development. Moreover, the GI tract of chickens contains villus cells, which act as the sites of nutrient digestion [28]. Al-Baadani et al. [29] reported that MOS, *Bacillus sp,* and synbiotics significantly increased Villus Height in the jejunum. Supplementation with prebiotics and synbiotics increased *Clostridium* sp. population numbers, which may have led to improvements in villi structure. Because *clostridium sp.* in the GI tract produced H2, they stimulate antioxidative enzymes such as selenoenzymes glutathione peroxidase and thioredoxin reductase to control reactive oxygen species, which reduces oxidative damage and protects epithelial cells from peroxidation, In addition, Clostridium is a lactate-utilizing bacterium, which converts acetic and lactic acid into butyric acid, Butyric acid promotes VH by regulation of gene ex pression and protein synthesis in villus cells [30]. Moreover, *Lactobacillus sp*. produced lactic acid, bacteriocins, bacteriostatics, and bacteriocides, which control the growth of pathogenic bacteria [31].

In terms of the VH: CD ratio in the small intestine, supplementing synbiotics significantly increased this ratio. Dizaji et al. [32] found that the supplementation with synbiotics (MOS mixed with B. subtilis) in diets significantly increased VH and the VH: CD ratio of the duodenum when compared with chickens fed diets supplemented with probiotics (Enterococcus faecium) or MOS alone. Under stress conditions, this indicates that supplementation of synbiotics clearly improves gut morphology and consequently increases nutrient utilization. The current study showed that the chickens fed di dietary prebiotic and synbiotic increased the number of goblet cells in the ileum. This is possibly due to the overgrowth of beneficial bacteria observed in the ileum that stimulated goblet cells and mucus secretion [33]. The higher VH/CD ratio in the synbiotic group indicates improved intestinal health and absorptive capacity, as supported by Cheng et al. [34], who demonstrated that this ratio is a reliable indicator of intestinal function and nutrient absorption efficiency.

The synergistic effect of prebiotics and probiotics in synbiotics creates an optimal environment for beneficial bacteria colonization, leading to better gut morphological development. The superior results observed in the synbiotic group compared to individual prebiotic or probiotic supplementation can be attributed to the complementary effects of these components.

## CONCLUSIONS

Synbiotic supplementation showed a better synergistic effect between prebiotics and probiotics than prebiotic and probiotic supplementation separately on performance, small intestine ratio, and small intestine histomorphology. Histomorphology of the small intestine and synbiotics significantly showed a trend towards efficient feed utilization, as seen in feed conversion.

## ACKNOWLEDGEMENT

The authors would like to thank the Faculty of Animal Science, Hasanuddin University, for permitting this research, especially the coordinators of the Laboratory of Animal Physiology and the Laboratory of Animal Production.

## AUTHORS' CONTRIBUTIONS

Conceptualized the study and drafted and revised the manuscript. All Author: Conducted experiments and analyzed data. All authors have read and approved the final manuscript.

## COMPETING INTERESTS

The authors have to declare that they have no competing interests.

## ETHICAL CLEARANCE

The authors have ethical clearance to use animals as research objects from the animal husbandry Faculty, Hasanuddin University.

## REFERENCES

[1] H. Yang, L. Paruch, X. Chen, A. van Eerde, H. Skomedal, Y. Wang, D. Liu, and J.L. Clarke, "Antibiotic Application and Resistance in Swine Production in China: Current Situation and Future Perspectives", Front. Vet. Sci., Vol. 6, p. 126, 2019. https://doi.org/10.3389/fvets.2019.00136

[2] G. Lhermie, Y.T. Gröhn, and D. Raboisson, "Addressing Antimicrobial Resistance: An Overview of Priority Actions to Prevent Suboptimal Antimicrobial Use in Food-Animal Production", Front. Microbiol., Vol. 7, p. 2114, 2017. https://doi.org/10.3389/fmicb.2016.02114

[3] B.M. Marshall and S.B. Levy (2011). "Food Animals and Antimicrobials: Impacts on Human Health", Clinical Microbiology Reviews, Vol. 24, No. 4, pp. 718–733, 2011. https://doi.org/10.1128/cmr.00002-11

[4] M. Arif, M.D. Akteruzzaman, T. Al-Ferdous, S.K.S. Islam, B.C. Bas, M.P. Siddique, and S.M.L. Kabir, 2022. "Dietary Supplementation of Bacillus-Based Probiotics on the Growth Performance, Gut Morphology, Intestinal Microbiota and Immune Response in Low Biosecurity Broiler Chickens", Veterinary and Animal Science, Vol. 14, p. 100216, 2021. https://doi.org/10.1016/j.vas.2021.100216

[5] W. Wang, H. Cai, A. Zhang, Z. Chen, W. Chang, G. Liu, X. Deng, W.L. Bryden, and A. Zheng, "Enterococcus Faecium Modulates the Gut Microbiota of Broilers and Enhances Phosphorus Absorption and Utilization", Animals, Vol. 10, No. 7, p. 1232, 2020. https://doi.org/10.3390/ani10071232

[6] T. He, S. Long, S. Mahfuz, D. Wu, X. Wang, X. Wei, and X. Piao, "Effects of Probiotics as Antibiotic Substitutes on Growth Performance, Serum Biochemical Parameters, Intestinal Morphology, and Barrier Function of Broilers", Animals, Vol. 9, No. 11, p. 985, 2019. https://doi.org/10.3390/ani9110985

[7] I. Gyawali, Y. Zeng, J. Zhou, J. Li, T. Wu, G. Shu, Q. Jiang, and C. Zhu (2022). "Effect of Novel Lactobacillus Paracaesi Microcapsule on Growth Performance, Gut Health and Microbiome Community of Broiler Chickens", Poultry Science, Vol. 101, No. 8, p. 101912. https://doi.org/10.1016/j.psj.2022.101912

[8] L.K. Froebel, S. Jalukar, T.A. Lavergne, J.T. Lee, and T. Duong, "Administration of Dietary Prebiotics Improves Growth Performance and Reduces Pathogen Colonization in Broiler Chickens", Poultry Science, Vol. 98, No. 12, pp. 6668–6676, 2019. http://dx.doi.org/10.3382/ps/pez537.

[9] M. Pourabedin, Z. Xu, B. Baurhoo, E. Chevaux, and X. Zhao, "Effects of Mannan Oligosaccharide and Virginiamycin on the Cecal Microbial Community and Intestinal Morphology of Chickens Raised Under Suboptimal Conditions", Canadian Journal of Microbiology, Vol. 60, No. 5, pp. 255–266, 2014. https://doi.org/10.1139/cjm-2013-0899

[10] C. Kridtayopas, C. Rakangtong, C. Bunchasak, and W. Loongyai, "Effect of Prebiotic and Synbiotic Supplementation in Diet on Growth Performance, Small Intestinal Morphology, Stress, and Bacterial Population Under High Stocking Density Condition of Broiler Chickens", Poultry Science, Vol. 98, No. 10, pp. 4595–4605, 2019. https://doi.org/10.3382/ps/pez152

[11] J.P. Wang, L. Yan, J.H. Lee, T.X. Zhou, and I.H. Kim, "Effects of Dietary Delta-Aminolevulinic Acid and Vitamin C on Growth Performance, Immune Organ Weight and Ferrum Status in Broiler Chicks", Livestock Science, Vol. 135, No. 2–3, pp. 148–152, 2011. https://doi.org/10.1016/j.livsci.2010.06.161

[12] H.A. Zulfa, D. Safira, T.A. Mawarni, and H.T. Saragih, "Effect of Ethanolic Extract of Wood Ear Mushroom on Growth Performance and Small Intestine Morphology of Jawa Super Chicken", Jurnal Veteriner, Vol. 22, No. 2, pp. 237–245, 2021. https://doi.org/10.19087/jveteriner.2021.22.2.237

[13] D.P. Rahardja, M. Yusuf, K.I. Prahesti, and V.S. Lestari, "Efficacy of Early Nutrition Programming for Improving the Performance of Kampung Chicken", European Journal of Veterinary Medicine, Vol. 2, No. 5, pp. 9–15, 2022. https://doi.org/10.24018/ejvetmed.2022.2.5.39

[14] D.P. Rahardja, M.R. Hakim, and V.S. Lestari, "Application of In Ovo Injection of L-Glutamine for Improving the Productivity of Indonesian Native Chicken: Hatchability and Hatching Time", IOP Conf. Ser.: Earth. Environ. Sci., Vol. 157, p. 012071, 2018.

[15] D. Song, A. Li, Y. Wang, G. Song, J. Cheng, L. Wang, K. Liu, Y. Min, and W. Wang, "Effects of Synbiotics on Growth, Digestibility, Immune, and Antioxidant Performance in Broilers", Animal, Vol. 16, No. 4, p. 100497, 2022. https://doi.org/10.1016/j.animal.2022.100497

[16] H. Ren, W. Vahjen, T. Dadi, E.M. Saliu, F.G. Boroojeni, and J. Zentek, "Synergistic Effects of Probiotics and Phytobiotics on the Intestinal Microbiota in Young Broiler Chicken", Microorganisms, Vol. 7, No. 12, p. 684, 2019. https://doi.org/10.3390/microorganisms7120684

[17] S.G.H. Tang, C.C. Sieo. K. Ramasmy, W.Z. Saad, H.K. Wong, and Y.W. Ho, "Performance, Biochemical, and Haematological Responses, and Relative Organ Weights of Laying Hens Fed Diets Supplemented with Prebiotic, Probiotic, and Synbiotic", BMC Vet. Res., Vol. 13, p. 248, 2017. https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-017-1160-y

[18] S. Dong, L. Li, F. Hao, Z. Fang, R. Zhong, J. Wu, and X. Fang, "Improving Quality of Poultry and its Meat Products with Probiotics, Prebiotics, and Phytoextracts", Poultry Science, Vol. 103, No. 2, p. 103287, 2024. https://doi.org/10.1016/j.psj.2023.103287

[19] Y.S. Liu, S. Li, X.F. Wang, T. Xing, J.L. Li, X.D. Zhu, L. Zhang, and F. Gao, "Microbiota Populations and Short-Chain Fatty Acids production in Cecum of Immunosuppressed Broilers Consuming Diets Containing ϒ-Irradiated Astragalus Polysaccharides", Poult. Sci., Vol. 100, No. 1, pp. 273–282, 2018. DOI: 10.1016/j.psj.2020.09.089.

[20] A.S. Anggraeni, A.E. Suryani, A. Sofyan, A.A. Sakti, L. Istiqomah, M.F. Karimi, and I.N.G. Darma, “Nutrient Digestibility of Broiler Chicken Fed Diets Supplemented with Probiotics Phytase-Producing”, IOP Conf. Ser..: Earth Environ. Sci., Vol. 462, p. 012003, 2010.

[21] N. Fathima, R.M. Rajendran, R. Mani, S. Balaji, and S. Vyas, “Nutritional Intervention with Bacillus Subtilis Strain PB6 in Early Days, Enhances Performance without Affecting Carcass Characteristics of Broiler Chickens”, Int. J. Vet. Sci. Res., Vol. 8, No. 3, pp. 100-109, 2022. DOI: https://dx.doi.org/10.17352/ijvsr.000121

[22] A. Biswas, K. Dev, P.K. Tyagi, and A. Mandal, “The Effect of Multi-Strain Probiotics as Feed Additives on Performance, Immunity, Expression of Nutrient Transporter Genes and Gut Morphometry in Broiler Chicken”, Anim. Biosci., Vol. 35, No. 1, pp. 64-67, 2023. DOI: 10.5713/ab.20.0749

[23] J. Marchewka, Sztandarski, Z.Z. Sasiadek, D.A. Urbanska, K. Damaziak, F. Wojciechowski, A.A. Riber, S. Gunnarsson, J. Marchewka, P. Sztandarski, and Z. Zdanowska-Sąsiadek, “Gastrointestinal Tract Morphometrics and Content of Commercial and Indigenous Chicken Breeds With Differing Ranging Profiles”, Animals, Vol. 11, No. 7, p. 1881, 2021. https://doi.org/10.3390/ani11071881

[24] D.P. Rahardja, M. Yusuf, K.I. Prahesti, and V.S. Lestari, “Efficacy of Early Nutrition Programming for Improving the Performance of Kampung Chicken”, European Journal of Veterinary Medicine, Vol. 2, No. 5, pp. 9-15, 2022. 10.24018/ejvetmed.2022.2.5.39

[25] Z. Alshamy, K.C. Richardson, H. Hünigen, H.M. Hafez, J. Plendi, and S. Al Masri, “Comparison of the Gastrointestinal Tract of a Dual-Purpose to a Broiler Chicken Line: A Qualitative and Quantitative Macroscopic and Microscopic Study”, PloS one, Vol. 13, No. 10, p. e020492, 2018. DOI: 10.1371/journal.pone.0204921

[26] M. Salmanzadeh and H.A. Shahryar, “Effects of Dietary Glutamine Addition on Growth Performance, Carcass Characteristics and Development at the Gastrointestinal Tract in Japanese Quails”, Rev. Med. Vet., Vol. 164, pp. 471-475, 2013.

[27] D.P. Rahardja, M. Yusuf, K.I. Prahesti, and V.S. Lestari, “Efficacy of Early Nutrition Programming for Improving the Performance of Kampung Chicken”, European Journal of Veterinary Medicine, Vol. 2, No. 5, pp. 9–15, 2022. https://doi.org/10.24018/ejvetmed.2022.2.5.39

[28] C.L. Ohland and C. Jobin, “Microbial Activities and Intestinal Homeostasis: A Delicate Balance Between Health and Disease”, Cellular and Molecular Gastroenterology and Hepatology, Vol. 1, No. 1, pp. 28–40, 2015. https://doi.org/10.1016/j.jcmgh.2014.11.004

[29] H. Al-Baadani, A. Abudabos, S. Al-Mufarrej, and M. Alzawqari (2016). “Effects of Dietary Inclusion of Probiotics, Prebiotics and Synbiotics on Intestinal Histological Changes in Challenged Broiler Chickens”, S. Afr. J. Anim. Sci., Vol. 46, No. 2, pp. 157-165. https://doi.org/10.4314/sajas.v46i2.6

[30] C. Kridtayopas, C. Rakangtong, C. Bunchasak, and Loongyai, “Effect of Prebiotic and Synbiotic Supplementation in Diet on Growth Performance, Small Intestinal Morphology, Stress, and Bacterial Population Under High Stocking Density Condition of Broiler Chickens”, Poultry Science, Vol. 98, No. 10, pp. 4595–4605, 2019. https://doi.org/10.3382/ps/pez152

[31] C. De Maesschalck, V. Eeckhaut, L. Maertens, L. De Lange, L. Marchal, C. Nezer, S. De Baere, S. Croubels, G. Daube, J. Dewulf, F. Haesebrouck, R. Ducatelle, B. Taminau, and F. Van

Immerseel, “Effects of Xylo-Oligosaccharides on Broiler Chicken Performance and Microbiota”, Appl. Environ. Microbiol., Vol. 81, No. 17, pp. 5880–5888, 2015. https://doi.org/10.1128/aem.01616-15

[32] B.R. Dizaji, S. Hejazi, and A. Zakeri, 2012. “Effects of Dietary Supplementations of Prebiotics, Probiotics, Synbiotics, and Acidifiers on Growth Performance and Organs Weights of Broiler Chicken. Euro. J. Exp. Bio., Vol. 2, No. 6, pp. 2125-2129, 2012.

[33] L. Wang, M. Lilburn, and Z. Yu, “Intestinal Microbiota of Broiler Chickens as Affected by Litter Management Regimens”, Front. Microbiol, Vol. 7, p. 593, 2016. https://doi.org/10.3389/fmicb.2016.00593

[34] Y. Cheng, Y. Chen, X. Li, W. Yang, C. Wen, Y. Kang, A. Wang, and Y. Zhoun, “Effects of Synbiotic Supplementation on Growth Performance, Carcass Characteristics, Meat Quality and Muscular Antioxidant Capacity and Mineral Contents in Broilers”, J. Sci. Food. Agric., Vol. 97, pp. 3699–3705, 2017. https://doi.org/10.1002/jsfa.8230