IMPACT OF DIETARY SYNBIOTIC SUPPLEMENTATION ON GROWTH PERFORMANCE, HAEMATOLOGICAL INDICES, AND INTESTINAL HEALTH OF BROILER CHICKENS CHALLENGED WITH *ESCHERICHIA COLI*

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Abstract: The experiment was conducted to investigate the impact of dietary synbiotic supplementation on the growth performance, haematological indices, and intestinal health of broiler chickens challenged with *E. coli*. One hundred 2-week-old Cobb 500 broiler chickens were randomly assigned and divided into four treatment groups, each with five replicates, containing five birds. The birds were housed for 35 days (before challenge) and 45 days (after challenge) with *E. coli*. The design for the dietary treatment supplementation is as follows: T1-basal diet, T2-2% *Bacillus licheniformis* in the basal diet, T3-2% *Saccharomyces cerevisiae* in the basal diet, and T4- 1% each of a combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* in the basal diet. The findings revealed that dietary synbiotic supplementation had no significant effect on feed intake, average weight gain, and feed conversion ratio in the treatment groups before and after broiler chickens were challenged with *E. coli* ($P > 0.05$). The results demonstrated a significant increase ($P<0.05$) in total protein and albumin in the synbiotic group. The synbiotic supplementation group shows a significant effect ($P<0.05$) on blood cell count, except basophils, which demonstrate no significant difference ($P>0.05$) between the groups. Villus height and crypt depth significantly increased ($P<0.05$) in the synbiotic group. Dietary synbiotic supplementation had a positive impact on growth performance, haematological indices, and intestinal health parameters before and after broiler chickens were challenged with *E. coli*.

Keywords: Broiler chickens, synbiotic, intestinal health, supplementation, haematological indices

1. Introduction

Feed additives, including probiotics, prebiotics, and synbiotics, can be used as an alternative treatment to replace antibiotics (Murarolli et al., 2014). The use of feed additives can improve host health by balancing the number of normal microflora, thereby enhancing mucosal barrier function and preventing the emergence of infections. The combination of probiotics and prebiotics can work in synergy to effectively inhibit pathogenic bacteria in the body of the host (Akutko & Stawarski, 2021; Ford et al., 2018). Thus, the fibre in prebiotics helps boost the bacteria of probiotics to work effectively. Synbiotic supplementation is a good antimicrobial growth promoter (AGP) which have been widely used in poultry in an attempt to improve broiler performance and to prevent pathogens and diseases (Acharya et al., 2024). Pathogenic bacterial infections such as *E. coli* in poultry cause high economic losses in the industry; therefore, there is a need to find solutions that are relatively cheaper and applicable to enhance poultry production.

S. cerevisiae is safe and widely applied to the feed sector, especially in feed fermentation. The use of *S. cerevisiae* culture can increase the amount of lactic acid bacteria, which will make the digestive tract environment acidic to inhibit the survival and growth of pathogenic bacteria (Ponomarova et al., 2017). It is a novel source as a focus on sustainability, cost, and scale emerges. On the other hand, *B. licheniformis* is a highly anaerobic spore-forming probiotic that can regulate the imbalance of the host's intestinal flora and maintain the microecological balance of the intestinal flora, fight pathogenic bacteria, promote the proliferation of beneficial intestinal bacteria, improve immunity, and overcome the imbalance (Pan et al., 2022).

In this study, we used *Bacillus licheniformis* strain and *Saccharomyces cerevisiae* (commercial baker's yeast) as the probiotic and prebiotic, respectively. Both have gained public attention with the surge in probiotic and prebiotic research. The banning of antibiotics as feed additives gives *B. licheniformis* and *S. cerevisiae* promising market prospects as a substitute for antibiotics because of their good performance in protecting animal health. Additionally, *B. licheniformis* and *S. cerevisiae* can effectively inhibit pathogenic infections with few side effects (Fazelnia et al., 2021). Moreover, both are also used in clinical treatment for pathogenic bacterial infections and intestinal microbiological disorders. In this study, a co-culture system of *B. licheniformis* and *S. cerevisiae* was used. In the co-fermentation system, the facultative anaerobic *B. licheniformis* strain consumes oxygen in the culture medium and provides an anaerobic environment for the absolute *S. cerevisiae*. Based on theory, the dietary synbiotic supplementation can work in synergy and support each other to enhance immunity and intestinal health. To further prove the potential of the combination of *B. licheniformis* and *S. cerevisiae* improving performance, histomorphology, and haematological parameters of broiler chickens, we investigated the effect of dietary synbiotic supplementation.

2. Materials and Method

2.1. Ethical approval

The experiment was performed in accordance with the regulations of the Universitas Jenderal Soedirman-LPPM, Indonesia, with the approval number 01.007/KEP-SAINTEK/VIII/2025. All the procedures were carried out at the experimental farm of the Faculty of Animal Science.

2.2. Experimental design and management

One hundred Cobb 500 broiler chickens were used. The chickens were randomly allocated into four treatment groups, with five replications, and each plot contained five chickens. The research treatment consists of: T1, Chickens that will not be given any feed additives in basal diet (control); T2, Challenge chickens were given *B. licheniformis* $5x10^9$ CFU 2g per 1000g of basal diet; T3, Challenge chickens that were given commercial *S. cerevisiae* of $1x10^{10}$ CFU 2g per 1000g of basal diet, T4, Challenge chickens that received the synbiotic combination of *B. licheniformis* and *S. cerevisiae* of 1g each per 1000g of basal diet**.** The *B. licheniformis* and *S. cerevisiae* (commercial baker's yeast) were used as probiotics and prebiotics, respectively. The birds were housed in twenty cages, which had adequate space to ensure easy movement. The birds were kept under controlled conditions under proper ventilation to maintain a constant temperature. The cages were cleaned on a daily basis to ensure proper sanitary living conditions. Throughout the experiment, the ambient temperature and relative humidity of the room were taken, with an average range of 26°C to 31°C and 60% to 88% respectively. The temperature and humidity range are not in the comfort zone for chickens; however, this is the daily condition from early morning to midday on the experimental farm and has been regulated using temperature, humidity, and wind speed controllers. The birds were kept under proper environmental, managerial, and hygienic conditions.

2.3. Feed and water

The birds were provided with water and feed *ad lib*. The chickens were fed a basal diet composed of commercial feed with the nutrient composition in **Table 1**, which provided the essential nutrients necessary to support the performance of the broiler chickens. The protein concentrate, derived from high-quality sources such as soybean and fish meal, ensured an optimal amino acid profile for the broiler chickens. The broiler chickens were vaccinated and provided with vitamin supplements, which were an integral part of the diet. Drinking water was supplemented per treatment and provided ad libitum to all chickens, with the supply continuously monitored to ensure it met the required standards for cleanliness and nutrient absorption. Additionally, prebiotics, probiotics, and synbiotics were supplemented when broiler chickens were two weeks old (Abdel-Hafeez et al., 2016). When chicks were two weeks old, they were given basal feed, vitamins, and the respective probiotics and prebiotics until day 35, at which point the chickens were challenged orally with *E. coli*. To induce challenge, chickens in the challenged group were given a single oral dose of 0.5 mL containing 1 × 10^6 CFU/mL *E. coli*. Chickens were monitored for symptoms such as fever, diarrhoea, loss of appetite, and thirst after administration of *E. coli* for up to 42 days.

Table 1. Nutrient composition of the basal diet

Feed Ingredient	**Proportion (%)**	**Protein (%)**	**Energy (Kcal/ Kg)**	**Lipid (%)**	**Fiber (%)**	**Ca (%)**	**P (%)**	**Lysin (%)**	**Meth (%)**
Corn	30	2,55	1005,00	1,14	0,66	0,006	0,0319	0,078	0.054
Rice	37,85	4,8	908,40	1,89	4,31	0,0264	2	0,2233	0,098
bran	8	3,5	178,40	25	49	95	0,2174	15	41
Soyabea	17,2	10,32	507,40	0,06	0,56	0,0232	5	0,2152	0,049
n	6	0	516,00	4	0,25	0,686	0,052	0,5332	6
Fish	0,25	0	0	2236	8	0	0,1111	0	0,170
meal	0,10	0,10	0	0	0	0,095	12	0	28
Oil	0,10	0,10	0	0	0	0	0	0,09	0
$CaCO_3$	0,20	0	0	0	0	0	0	0	0
L-lysin	0,30	0	0	0	0	0,0001	0	0	0
DL-Met	**100**	**21,46**	**3115,2**	0	0	2	0	0	0,09
Top mix		**39**		0	0	0	0,0003	**1,1397**	0
NaCl				**5,33**	**5,79**	**0,8368**	8	**15**	0
Total				**25**	**29**	**15**	0		**0,462**
							0,4111		**29**
							57		

Growth Performance

At 35th and 45th days, before and after chickens were challenged with *E. coli*. Before and after broiler chickens were challenged with *E. coli*, the basic productive performance indicators were measured during the experimental period, which included average daily gain (ADG), feed intake, and feed conversion efficiency (FCR).

Haematological parameters

Blood samples were drawn from the wing vein, using sterile syringes, and transferred into anticoagulant-treated tubes for plasma separation. On day 35, five chickens per treatment, with 3 mL each, were randomly selected for haematological analysis and serum extraction for subsequent analysis. Determination of total protein (TP), albumin, fibrinogen, lymphocytes, heterophils, basophils, eosinophils, and packed cell volume (PCV) was carried out.

Histological Investigation

A total of five chickens per treatment were euthanized, and samples of the intestine (the tube before Meckel's diverticulum) were excised and rinsed with 0.9% saline to remove all contents. The various tissue sections were fixed in 10% buffered formalin, embedded in paraffin wax blocks, sectioned at 5µm thickness, mounted on glass slides, and stained with Hematoxylin and Eosin. The morphometric parameters were villous height (VH: from the tip of the villi to the crypt), crypt depth (CD: from the base of the villi to the submucosa). A light microscope with a 10x or 40x objective lens equipped with a digital image analyzer was used to measure the villus length and crypt depth in the broiler chickens. The image analyzer software (ImagePro Plus) was used to quantify the villus height and crypt depth.

Statistical Analysis

The data obtained in the experiment were statistically analyzed using a one-way ANOVA test, SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics helped in the data analysis; the means and standard deviation were calculated. Differences between treatments were examined using the Duncan test to determine the significance level (P<0.05). The Graph Pad Prism was used to design the graphs.

3. Results

Growth performance

The results of feed intake, body weight, and feed conversion ratio (FCR) are presented in **Table 2**. From the results, feed intake, AWG, and FCR showed no significant difference (P>0.05) in the treatment groups before and after broiler chickens were challenged with *E. coli*.

Table 2. Effect of dietary synbiotic supplementation on growth performance before and after broiler chickens were challenged with E. coli.

Growth Performance	T1	T2	T3	T4	P value
35-day-old-chicks					
Feed intake (g)	3348.1±601.4	3759.8±561.9	4076.5±519.4	4084.5±645.2	0.193
AWG (g)	2267.2±264.8	2511.3±487.2	2787.6±438.4	2586.5±332.1	0.249
FCR (g)	1.5 ±0.11	1.5±0.10	1.5±0.05	1.6±0.06	0.217
42-day-old-chicks					
Feed intake (g)	3463.7 ±523.4	4086.8 ±1050.1	4260.2±395.3	3858.2±12233	0.521
AWG (g)	2121.6 ±269.1	2379.6 ±751.0	2580.7 ±495.6	2312.9±696.2	0.671
FCR (g)	1.6 ±0.18	1.8±0.36	1.7±0.21	1.7±0.15	0.851

Notes: T1-control, T2-*Bacillus licheniformis* group, T3- *Saccharomyces cerevisiae* group, and T4- synbiotic group.

The findings presented in **Table 3 and Fig. 1 indicate that dietary supplementation with synbiotic significantly increased (P <** 0.05) the length of villi and crypt depth compared to the control groups, both before and after the broiler chickens were challenged. Before the broiler chickens were challenged, the results showed that there was no significant difference (P > 0.05) in the villus height among the treatment groups. The crypt depth showed no significant difference (P>0.05) between T2 and T3, and no significant difference (P>0.05) between T3 and T4. On the other hand, after broiler chickens were challenged, the treatment groups showed a significant difference (P<0.05) in T2, T3, and T4 of the crypt depth and villus height. However, the crypt depth showed no significant difference (P>0.05) between the control group and T2.

Table 3. Effect of dietary synbiotic supplementation on crypt depth and villus length in the intestine of broiler chickens before and after being challenged with *E. coli*

Treatment	**Crypt depth(µm) 35-day-old-chicks**	**Villi length(µm)**	**Crypt depth(µm) 42-day-old-chicks**	**Villus length(µm)**
T1	215.0 ± 29.0^{a}	631.41 ± 18.6^{a}	287.0 ± 1.5^{a}	613.5 ± 34.2^{a}
T2	236.0 ± 26.0^{ab}	812.9 ± 22.0^{b}	333.9 ± 17.0^{a}	726.3 ± 45.3^{b}
T3	257.6 ± 23.4^{bc}	828.1 ± 43.5^{b}	293.9 ± 4.1^{b}	694.0 ± 90.7^{c}
T4	271.9 ± 12.0^{c}	842.8 ± 44.3^{b}	358.7 ± 6.6^{c}	928.7 ± 56.2^{d}
P value	0.003	< 0.001	< 0.001	< 0.001

[a,b,c,d] Data in the same row with different superscripts are significantly different ($P<0.05$). T1-control, T2-*Bacillus licheniformis* group, T3- *Saccharomyces cerevisiae* group, and T4- synbiotic group.

Figure 1 shows the morphology of the intestine, showing the villus length and crypt depth before and after broiler chickens were challenged with *E. coli*.

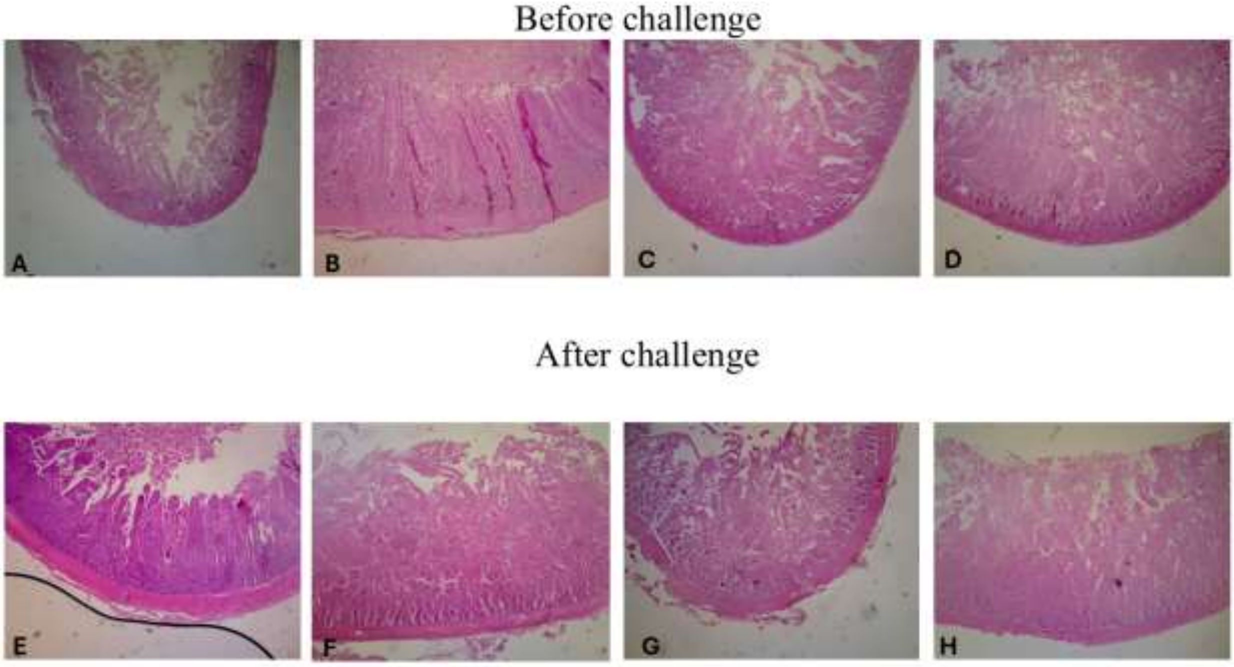
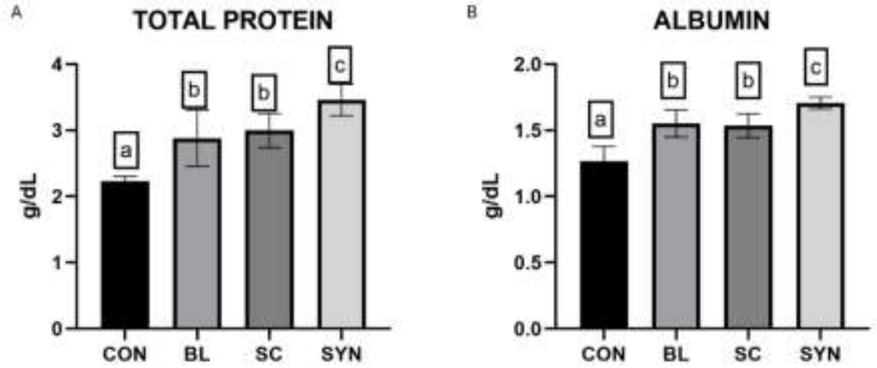


Fig. 1 Histology of the intestine before and after being challenged with E. coli. A&E-control, B&F- *Bacillus licheniformis* group, C&G- *S. cerevisiae* group, and D&H- synbiotic group

The results in **Fig. 2** showed the effect of dietary synbiotic supplementation on blood protein. The findings demonstrated a significant increase ($P<0.05$) in blood protein levels (Total protein, fibrinogen, and albumin) in the synbiotic group. The albumin level showed a significant difference ($P<0.05$) in T2, T3, and T4 compared to the control. However, there was no significant difference ($P>0.05$) between T2 and T3. The total protein level showed no significant difference ($P>0.05$) between T2 and T3 compared to the control. The fibrinogen levels of T2 and T3 showed no significant difference ($P>0.05$) compared to T1 and T4.

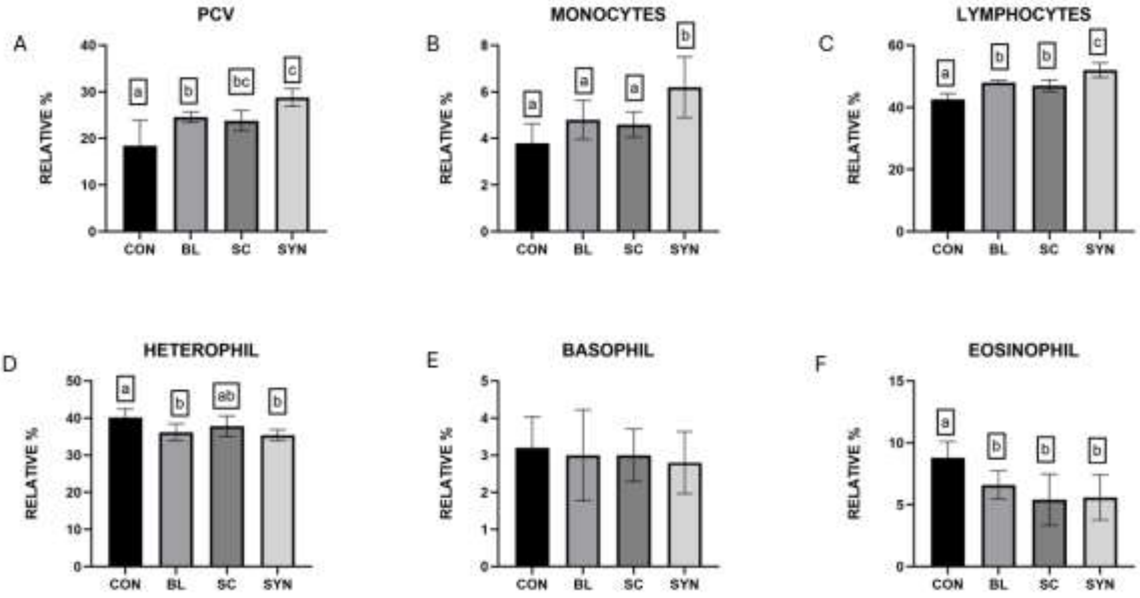
Figure 2. Effect of dietary synbiotic supplementation on the blood protein of broiler chickens



BL: *B. licheniformis*, SC: *S. cerevisiae*, mix: synbiotic combination of *B. licheniformis* and *S. cerevisiae*. Data are with the means ± SEM (n = 4). $^{a\text{-}c}$ Means with different letters are significantly different (P <0.05).

The findings presented in Fig. 3 indicate that dietary synbiotic supplementation had a significant effect (P<0.05) on blood cells, except basophils, which showed no significant difference (P>0.05) between the groups. Compared to the control groups, the blood cell counts (PCV, monocytes, lymphocytes, heterophils, and eosinophils) showed a significant difference (P<0.05) in the synbiotic groups. The heterophil and eosinophil counts did not show any significant difference (P>0.05) between the treatment groups. The findings further revealed that PCV and lymphocytes showed no significant difference (P>0.05) between T2 and T3. The monocyte count compared to the control showed no significant difference with T2 and T3.

Figure 3. Effect of dietary synbiotic supplementation on blood cell counts of broiler chickens



BL: *B. licheniformis*, SC: *S. cerevisiae*, mix: synbiotic combination of *B. licheniformis* and *S. cerevisiae*. Data are with the means ± SEM (n = 4). $^{a\text{-}c}$ Means with different letters are significantly different (P <0.05).

4. Discussion

This study demonstrates that synbiotic supplementation did not significantly stimulate body weight, feed intake, and FCR. This is not in accordance with the findings of (Duff et al., 2020; Nawaz et al., 2025), where synbiotic feed supplementation significantly impacted the growth performance of broilers after exposure to infection. The present findings (**Table 2**) demonstrate that the dietary supplement feed additives had beneficial effects on broiler chickens' performance. The feed conversion results showed that, although there was no significant difference in body weight, feed intake influenced the increase in body weight, thereby improving the efficiency of feed utilization. The improvement of FCR in the study is likely to improve nutrient and energy utilization. The *Bacillus licheniformis* (present in the synbiotics) population might have increased in the intestines of broiler chickens, has been progressively recognized as a significant factor impacting their health and growth performance. Probiotics can dwell well in the digestive system with the help of prebiotics, both exhibiting a synergy, which will enable them to tolerate an anaerobic environment, that is, an environment with low pH, temperature, and oxygen. Furthermore, the mannan and beta-glucan components in the yeast wall are utilized in conjunction with probiotic bacteria, demonstrating a prebiotic effect through their ability to enhance the growth, metabolism, and/or beneficial activities of probiotics, ultimately resulting in desirable performance traits.

The impact of dietary synbiotic supplementary treatment had a significant effect on haematological indices. Haematological parameters are valuable indicators of the immune response and overall health status of broiler chickens; any changes can reveal physiological effects of infections, stress, nutrition, and vaccination on the immune system. A study in which broilers were exposed to Clostridium perfringens found improvements in haematological parameters when treated with synbiotics (Al-Baadani et al., 2018). As depicted in **Fig. 2**, the protein level (total protein and albumin) was significantly higher in the synbiotic group. This is in contrast with (Żbikowski et al., 2020) who reported no correlation with total protein and albumin of chicken blood in the synbiotic supplemented group. Proteins play a crucial role in regulating water balance in the body, helping to distribute body fluids evenly between the blood and body tissues. Albumin and proteins play a major role in the deposition of protein in meat. This is in accordance with (Liu et al., 2015), which indicates that albumin affects the growth rate of broiler chickens. However, low albumin will affect the total protein. Albumin supports immune function and can be an indicator of the overall health and nutritional status of broiler chickens (Tóthová et al., 2019). Dietary supplementation with synbiotics increased total protein in this study, indicating that synbiotics can improve protein anabolism in broilers. Synbiotics can mitigate inflammation, promoting the growth of beneficial gut bacteria that produce anti-inflammatory short-chain fatty acids, thereby preserving anabolism (Al-Habsi et al., 2024).

Prior studies have demonstrated that synbiotics influence red blood cells (RBCs) and white blood cells (WBCs), tending to improve their counts, which is a similar trend to the present findings (Ellakany et al., 2011; Nawaz et al., 2025; Żbikowski et al., 2020). However, in the present findings, the count of the PCV and WBCs in all the treatment groups is within the normal range. This implies that the broiler chickens are in a healthy condition; thus, their immune system is functioning properly. PCV helps to diagnose anaemia, giving insights into chickens' physiology and immune responses. However, the finding of (Matthew et al., 2022) demonstrated that the PCV concentration increased in the synbiotic-supplemented group of broiler chickens challenged with *Eimeria tenella*. Furthermore, in their study, the PCV concentration decreased as early as 4 days post challenge in the control group. This is not unrelated to the severe acute hemorrhages consistent with caecal coccidiosis, which caused loss of blood components (Akhtar et al., 2015; Melkamu et al., 2018). The lymphocytes and heterophils in the present study showed the highest count in the synbiotic-supplemented group may be ascribed to the induction of an immune response as a defence mechanism against *E.*

coli. Lymphocytes play a crucial role in the formation of antibodies that circulate in the blood, as well as in the development of the immune system. Since the primary function of lymphocytes is immunological response, humoral antibody production, and cell-mediated immunity, chronic antigenic stimulation can result in a greatly expanded circulation lymphocyte pool (Chi et al., 2024). Heterophile plays a critical role in innate immunity by defending the chickens against microbial pathogens through the release of cytokines and chemokines, which are signalling molecules that help coordinate the immune response (Broom, 2019). The monocyte counts in this study showed the highest count in the synbiotic group compared to the control group, which is in agreement with the finding of (Sunu et al., 2021). Monocytes are phagocytic cells that convert into macrophages once they penetrate tissues (Patel et al., 2021). Furthermore, monocytes and macrophages communicate through chemotaxis, which entails migrating towards an inflammatory gradient (Hall et al., 2024). Most of the inflammatory tissues of macrophages have similar functions, like surveillance, removal of dead cells and cellular debris, defence against pathogens, promotion of wound healing and tissue remodelling, and repair (Lech et al., 2012).

One of the indicators of the birds' health and the health of the intestines in broiler chickens is the small intestine histology, which consists of villus height and crypt depth. These two are directly coupled with enhanced epithelial turnover, and longer villi are associated with cell mitosis. This study has identified that synbiotic supplementation showed a positive effect on villus length and crypt depth before and after chickens were challenged with *E. coli*. It is possible to suggest that the synbiotics reduced the number of *E. coli* in the intestine, which impacted the crypt and villi. However, this will increase the surface area of the intestine to improve the absorption of nutrients. In this study, we can suggest that the administration of synbiotics with the presence of *Bacillus licheniformis* (bacteria) colonized the intestine successfully, shielding the villi from harmful bacteria (*E. coli*), which could account for improving intestinal morphology (Šefcová et al., 2023). Synbiotics enhance the gut environment, improve digestion and nutritional absorption, while reducing pathogenic infection damage and increasing the bioavailability of vital minerals. The villus and crypt are also known as the villus factory, and deeper crypts show fast tissue turnover to permit renewal of the villus as needed in response to normal inflammation from pathogens or their toxins in high-demand tissue (Awad et al., 2009; Sobotik et al., 2021). In this study, we suggest that a wide absorptive surface area enables a stronger expression of brush border enzymes and nutrient carriage systems, resulting in improved digestion and absorption capabilities of the intestine (Azman et al., 2022). It is, however, believed that increasing villus height is correlated with these processes.

5. Conclusion

Dietary synbiotic supplementation containing *B. licheniformis* and *S. cerevisiae* (commercial baker's yeast) positively impacted the growth performance, haematological indices, and intestinal health parameters before and after broiler chickens were challenged with *E. coli*. The synergistic effect of *B. licheniformis* and *S. cerevisiae* increased the villus length and crypt depth, blood protein (albumin and total protein), blood cell count (PCV, monocytes, and lymphocytes), and enhanced the performance traits of broiler chickens in the present findings.

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