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Effects of the Combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* on Blood Profile, Immune Organs, Carcass Characteristics, and Intestinal Health of Broiler Chickens Challenged with *Escherichia coli*

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ABSTRACT

The poultry industry faces ongoing challenges from bacterial infections. Probiotics have emerged as a promising strategy to improve the performance and health of animals. The current research aimed to evaluate the effectiveness of the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* on blood profile, immune organs, carcass characteristics, and intestinal health in broiler chickens exposed to *Escherichia coli* (*E. coli)*. This study involved the random assignment of 100 male Cobb 500 broiler chicks aged one day, raised for 35 days, each with an average weight of 44.26 ± 1.89g, to four oral treatments. There were four groups, each group consisting of five replications, and each replication consisting of five chickens. T1, the control group, received a basal diet. T2 received *B. licheniformis* (5×10^9 CFU per 2g), administered at a level of 2g per 1000g of basal diet. T3 received *S. cerevisiae* (1.0×10^{10} CFU per 2g), administered at a level of 2g per 1000g of basal diet. T4 received a combination of *B. licheniformis* (5×10^9 CFU/g) and *S. cerevisiae* (1.0×10^{10} CFU/g), with each probiotic administered at a level of 1g per 1000g of basal diet. The data of blood profile parameters, including electrolytes, leukocytes, total protein of plasma (TPP), fibrinogen, hemoglobin, LDL, HDL, triglycerides, and cholesterol, indicated a notable disparity between the control group and the group receiving the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*, with the control group exhibiting lower values for these parameters compared to the combination group. Total bacteria counts before and after the challenge showed fewer colonies of *E. coli* in the group that received the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*. The weights of carcass parts (breast, wing, and thigh) and immune organs (spleen, Bursa Fabricius, and intestine) were all significantly lower in the control group compared to the group administered a combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*. These results indicated that the supplementation of *Bacillus licheniformis* and *Saccharomyces cerevisiae* to broiler chickens exposed to *E. coli* increased their parameters of blood profile, immune system, carcass features, and intestinal health.

Keywords: *Bacillus licheniformis*, Broiler chickens, Immune function, Probiotics, *Saccharomyces cerevisiae*

INTRODUCTION

Broiler chicken is widely consumed as human population growth contributes to the rapid increase in the consumption of meat. There are several challenges facing poultry production processes, and one of these includes diseases, which are a major threat to broiler chicken production (Gržinić et al., 2023). Over the past decade, preventative measures, like antibiotics and vaccinations, have been utilized to manage bacterial infections. However, these preventive measures are no longer in use because synthetic antibiotics are prohibited in the livestock industry, and the prevalence of chemical contaminations in animal products is common (Ghimpețeanu et al., 2022). The discontinuation of antimicrobial growth promoters (AGPs) has made poultry more susceptible to infectious diseases, negatively impacting production efficiency (Abreu et al., 2023). Several mechanisms have been proposed to prevent or reduce pathogenic infections in poultry, including modulating gut microbiota composition, enhancing the barrier surrounding the intestines, and boosting antibodies (Di Vincenzo et al., 2024). The increasing need for sustainable and effective solutions has driven the search for alternative approaches to poultry production. Feed additives such as probiotics and prebiotics provide a long-term and efficient way to boost the production and health of chickens. Additionally, they are commonly used in commercial settings. Probiotics and prebiotics can enhance broiler chicken health by modifying the gut microbiota and fortifying the gut barrier (Dong et al., 2024). These microorganisms can modulate the gut microbiota to improve host health through mechanisms such as acidification, immune stimulation, pathogen inhibition, and the reduction of harmful bacteria levels in the gut.

Bacillus licheniformis (*B. licheniformis*) is a bacterium recognized as harmless and has been widely utilized in the livestock sector (Elleithy et al., 2023). *Bacillus licheniformis*, a promising probiotic strain, exhibits several beneficial

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properties, such as spore-forming ability, resistance to harsh conditions, and the production of antimicrobial substances (Ramirez-Olea et al., 2022). These attributes make it a valuable alternative for enhancing animal health and productivity. The strain is used to produce a polypeptide antibiotic known as bacitracin (Zhu et al., 2023). *B. licheniformis* has the potential to produce bacteriocin under aerobic conditions, as well as under anaerobic conditions against anaerobic microorganisms (Shleeva et al., 2023). Among the *Bacillus* species, *B. licheniformis* was found to have an antipathogenic action in the gastrointestinal tract of broilers (Chen and Yu, 2020). *B. licheniformis* has been documented to synthesize many biologically active compounds, including digestive enzymes, lysozymes, bacteriocins, and antimicrobial peptides. They are acknowledged for augmenting animal performance through the enhancement of feed digestibility, stimulation of immune system development, improvement of intestinal mucosal barrier function, inhibition of pathogenic bacterial colonization, promotion of beneficial microorganisms proliferation, and maintenance of intestinal microflora equilibrium (Chen and Yu, 2020; Kan et al., 2021). In broilers, *B. licheniformis* may be a promising growth booster of the intestinal balance of the microbial population. It possesses significant promise for enhancing the performance and productivity of poultry. Therefore, in broiler chickens raised for commercial settings, the application of *B. licheniformis* spores as direct-feed microorganisms or probiotics may be a viable substitute for antibiotics in preventing and treating harmful bacteria (Kan et al., 2021).

Saccharomyces cerevisiae (*S. cerevisiae*; baker's yeast) has garnered considerable interest as an alternative to antibiotics due to its prebiotic and probiotic properties. As the primary components, *S. cerevisiae* comprises beta-glucans and mannan-oligosaccharides (MOS). In combination, beta-glucan and MOS from the yeast cell wall may enhance intestinal development, increase poultry performance, and strengthen the intestinal ecology in broiler chickens by boosting immunity, which protects against pathogenic infection (Nikpiram et al., 2013; Teng et al., 2021). A variety of studies have shown that *S. cerevisiae* is an alternate protein source that improves the immunological response, blood parameters, and growth potential of the chicken capacity of poultry (Qui, 2023). Additionally, yeast serves as an outstanding source of short peptides that include free amino acids. This guarantees a swift process of absorption and digestion, potentially improving feed efficiency substantially. In chicken diets, yeast is used as a prebiotic and probiotic to stimulate the release of bile acids. It is utilized in the recovery of acid bile, leading to an increased production of cholesterol as a precursor of acid bile. It assists in the reduction of blood serum cholesterol levels as a precursor of acid bile (Azrinnahar et al., 2021). Yeast exhibits antibacterial characteristics that contribute to the host's immunomodulatory response. Mycocins are produced by yeast to defend against pathogenic bacteria. These mycocins secrete inhibitory substances that degrade toxins, preventing the adhesion of pathogens to the epithelial cell surface and creating competition for nutrition (Hatoum et al., 2012).

Probiotics have been reported to improve the metabolism and physiology of food animals (Abd El-Hack et al., 2020; Anee et al., 2021). The combining effect of different probiotic strains may be complementary or synergistic. Complementary probiotics function independently, with each strain contributing its benefits to the host (Cunningham et al., 2021). For instance, one strain may enhance nutrient absorption while another may improve gut barrier function. Synergistic probiotics work together to achieve a greater effect than the sum of their contributions. In this case, the strains may interact to produce metabolites that benefit the host or may compete with pathogens for resources. Furthermore, the future of probiotic research will be influenced by the development of novel strains. These strains should be specifically selected and targeted to fill unoccupied niches within the individual's microbiome. This approach has the potential to expand the applications of probiotics beyond the gastrointestinal tract, including ex-gut sites. For example, in poultry, probiotics can positively impact respiratory health. However, studies on the effects of the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* on immunity and gut health are limited. This study was motivated by the lack of research and the inconsistent results to evaluate the hypothesis that the combination of *B. licheniformis* and *S. cerevisiae* could improve the intestinal health and blood profile of broiler chickens, supporting the use of these probiotics in place of antibiotic growth promoters (AGP) in broiler diets.

MATERIALS AND METHODS

Ethical approval

Every producer of animals used in this study complied with the ethical standards number 021/EA/PDHI/XI/2024 of the Association of Indonesian Veterinary (PHDI) in Central Java Branch, Indonesia, in addition to national and institutional regulations regarding animal care and usage. The slaughtering and collection of blood samples were carried out as per the standard sampling procedure for experimental purposes.

Experimental animals and treatment

A total of one hundred Cobb 500 broiler chickens were used in this 35-day research carried out at the experimental farm, Faculty of Animal Science, Universitas Jenderal Soedirman, Indonesia. The chickens used for this research were one day old, with an average body weight of 44.26 + 1.89 g from a bonafide broiler chicken company in Central Java,

Indonesia. There were four treatment groups to which the poultry were randomly assigned, each of which contained five chickens, and in five replications. The research treatments consisted of four groups: T1, the control group, received a basal diet. T2 received *B. licheniformis* at a dose of $5x10^{9}$ CFU per 2g, administered at a level of 2g per 1000g of basal diet. T3 received *S. cerevisiae* at a dose of $1,0\ x10^{10}$ CFU per 2g, administered at a level of 2g per 1000g of basal diet. T4 received a combination of *B. licheniformis* $5x10^{9}$ CFU/g feed and *S. cerevisiae* $1,0 \times 10^{10}$ CFU/g feed, with each probiotic administered at a level of 1g per 1000g of basal diet.

Housing and management

All chickens were housed in twenty cages to facilitate precise monitoring and feeding. Each cage plot had a size of 1 m2, and this allowed adequate space for the chickens' movement while ensuring the necessary containment for feed and water intake. The poultry were maintained under identical environmental, managerial, and hygienic conditions. Throughout the experiment, daily readings of the room's relative humidity and ambient temperature were taken; the averages ranged from 60% to 88% and 26°C to 31°C, 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. During the research, chickens were given lighting ranging from 23-24 hours every day, with a light intensity of 25 lux.

Feed and water

The nutritional content of Cobb 500 broiler feed ingredients is shown in Table 1. Chickens are given basal feed with a protein composition of 21.46%, EM 3115.2 kcal/kg feed (Table 2). This provides essential nutrients to support the performance of broiler chickens (Ofori et al., 2019). Broiler chickens are first vaccinated with ND by the hatchery company before being sent to the research site. As soon as the chickens arrive in the coop, they are immediately given sugar water and starter feed. On the second day until harvest, chickens are given Topmix supplements produced by PT Medion Farma Jaya, which contain multivitamins, minerals, and amino acids of 5 grams per kg of feed. Additionally, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* were supplemented when broiler chickens were eight days old until 28 days old. At 29 days old, the chickens in all treatment groups were challenged with a single oral dose of 0.5 mL 1×10^{6} CFU/ml *E. coli* (Eid et al., 2022). Water was provided *ad libitum* to all chickens, with the supply continuously monitored to ensure it met the required standards for cleanliness and nutrient absorption. After the age of 29 to 35 days following the administration of *E. coli*, chickens were observed for symptoms such as fever, diarrhea, loss of appetite, and dehydration.

Table 1. Nutrient content of Cobb 500 broiler chicken feed ingredients

Feed Ingredient	Protein (%)	Energy (Kcal/kg)	Lipid (%)	Fiber (%)	Ca (%)	P (%)	Lysin (%)	Met (%)
Corn	8,5	3350	3,8	2,2	0,02	0,28	0,26	0,18
Rice bran	12,9	2400	5	11,4	0,07	1,5	0,59	0,26
Soybean meal	44	2230	0,8	7	0,29	0,65	2,69	0,62
Fish Meal	60	2950	13	1,5	3	1,7	3,1	0,99
Oil	0	8600	0	0	0	0	0	0
CaCO3	0	0	0	0	38	0	0	0
L-lysin	95,6	0	0	0	0	0	90	0
DL- Met	58,6	0	0	0	0	0	0	90
Topmix	0	0	0	0	0,06	0,5	0	0
NaCl	0	0	0	0	0	0	0	0

Ca: Calcium; P: Phosphor

Table 2. Nutrient content of basal feed

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	0,03192	0,078	0,054
Rice bran	37,85	4,88	908,40	1,8925	4,3149	0,026495	0,215745	0,223315	0,09841
Soybean Meal	8	3,52	178,40	0,064	0,56	0,0232	0,052	0,2152	0,0496
Fish Meal	17,2	10,32	507,40	2,236	0,258	0,686	0,111112	0,5332	0,17028
Oil	6	0,00	516,00	0	0	0	0	0	0
CaCO3	0,25	0,00	0,00	0	0	0,095	0	0	0
L-lysin	0,1	0,10	0,00	0	0	0	0	0,09	0
DL- Met	0,1	0,10	0,00	0	0	0	0	0	0,09
Topmix	0,2	0,00	0,00	0	0	0,00012	0,00038	0	0
NaCl	0,3	0,00	0,00	0	0	0	0	0	0
	100	21,4639	3115,2	5,3325	5,7929	0,836815	0,411157	1,139715	0,46229

Ca: Calcium; P: Phosphor

Data collection

On day 35, 3 ml blood samples were collected from 20 chickens, with five chickens per treatment for routine hematology analysis. A routine hematology analysis is a basic assessment of several blood components. Starting from the erythrocyte (red blood cell) component to the leukocyte (white blood cell) component. Using sterilized syringes, samples of blood were obtained from the wing vein and transferred to tubes containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for plasma separation. After the Islamic slaughter (performed by a Muslim slaughterer method, and ensuring a swift, humane cut to the jugular vein, carotid arteries, and windpipe to ensure complete exsanguination), the lymphoid organs, which included the intestine, spleen, and Bursa Fabricius, were assessed at 35 days of age.

Blood profile analysis

Centrifugation was used to separate the blood plasma, which was then kept at -20°C for subsequent analysis for ten minutes at 3000 rpm. A Seamaty SD1 biochemistry machine with standard biochemical kits and an automated analyzer were used to measure electrolytes, leucocytes, fibrinogen, hemoglobin (Hb), total protein plasma (TPP), total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in the blood samples.

Microbiological enumeration

Two chickens were euthanized using Natrium pentobarbital from each cage at 28 and 35 days, and samples were obtained from the duodenum, jejunum, ileum, and cecum. Bacteriological examination was conducted on all samples, which were stored at 4°C until further investigation. For the analysis of the samples, 1g of the digested contents of each sample was diluted with 0.1% peptone water and thereafter prepared for serial dilution. The total bacterial count for *E. coli* would be determined by spreading 50µl of the serially diluted tubes onto EMB agar. At 39°C, each plate was incubated for a full day. Ultimately, the colony counts were given as the mean 10-logarithm colony-forming units (log_{10}CFU) per gram of sample content. A count of *E. coli* was performed using the methodology of Jazi et al. (2019).

Statistical analysis

The acquired data were organized with Excel (Microsoft); the data were subjected to one-way ANOVA analysis with SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and presented as least squares mean ± standard deviation. Differences between treatments were examined using Duncan's multiple tests. Significance levels are indicated as $P < 0.05$ *, $P < 0.01$**, and $P < 0.001$***.

RESULTS

In Table 3, the blood profile is illustrated by the impact of dietary *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and the combined effect of *Bacillus licheniformis* and *Saccharomyces cerevisiae*. When compared to the other groups, the T4 group had considerably higher levels of hemoglobin, fibrinogen, and electrolytes ($p < 0,05$). Although the T1 and T3 groups did not exhibit any significant differences, the total protein plasma of the T1, T2, and T4 groups did ($p < 0.05$). Additionally, all of the groups' leukocyte levels varied significantly, with T2 having the highest level of leukocytes ($p < 0.05$). Nevertheless, the control group's cholesterol level was noticeably greater than that of the T2, T3, and T4 groups ($p < 0.05$). On the other hand, triglycerides showed significant differences in all the groups compared with the T4 group, which recorded low levels of triglycerides ($p < 0.05$). In comparison to the other groups, the control group exhibited substantially higher levels of LDL and HDL ($p < 0.05$).

Table 4 shows the results for the total bacteria population of *E. coli* in the intestine before the chickens were challenged with *E. coli*. The control group recorded higher colonies of *E. coli* found in all parts of the intestine. Compared to the T1, T2, and T3 groups, the T4 group was noted to have a lower number of *E. coli* in all parts of the intestine (duodenum, jejunum, ileum, and cecum) ($p < 0.05$). Moreover, the statistical analysis revealed no discernible changes between the control group and the other groups in the duodenum and the ileum. However, a significant difference existed between T2, T3, and T4 ($p < 0.05$). There were no notable variations between the jejunum of the control group and T4 ($p < 0.05$).

Table 5 demonstrates the overall population of *E. coli* bacteria in the intestine following exposure to *E. coli*. The results presented similar findings to those in Table 4, with the control group having a higher number of colonies in the various parts of the intestine. In addition, the T1 group's duodenum showed no discernible differences in T2, T3, or T4, whereas T2, T3, and T4 showed significant differences ($p < 0.05$). In the jejunum, there is a substantial difference across all groups, except for T1 and T4, which did not exhibit any significant difference. Significant changes in T2, T3, and T4 were seen in the ileum ($p < 0.05$). While there was a substantial change between T1 and T2, there was no discernible variation in the cecum between the control groups T3 and T4 ($p < 0.05$). Following the broiler chickens' exposure to *E. coli*, the study's findings indicated that the number of *E. coli* had increased.

Table 6 shows the result of the weight values of the T4 groups, which were higher than those of the other groups in both carcass weight and immunological organs ($p < 0.05$). Nonetheless, the weight of the wing and thigh exhibited a

substantial difference across all groups ($p < 0.05$). While there was no discernible change in the breast weight between T1 and T4, as well as between T3 and T4, there was a substantial difference in breast weight between the T1, T2, and T3 groups ($p < 0.05$). The intestine exhibited a substantial variation in weight across all groups as compared to the T4 group, which demonstrated a larger weight value than the others ($p < 0.05$). Bursa Fabricius exhibited a notable difference among the T1, T2, and T3 groups; however, when compared to T4, no significant differences were observed across all groups. Furthermore, the T1 group exhibited a reduced spleen weight than the other groups; however, this was not statistically different ($p < 0.05$). Conversely, the T2, T3, and T4 groups exhibited substantial differences ($p < 0.05$).

Table 7 shows the results for carcass weight and immune organs after the chickens were challenged with *E. coli*. The results showed significant differences in breast weight and thigh weight for the carcass weight in all the groups ($p < 0.05$). However, the control group showed the lowest weight value amongst all the groups in breast weight, thigh weight, and wing weight ($p < 0.05$).

The control group exhibited significant differences in intestine weight compared to the T2, T3, and T4 groups after the chickens were challenged with *E. coli* ($p < 0.05$). Although there was no discernible difference in the spleen between the control group and the other groups, the T4 group had the largest spleen weight, and the control group had the lowest ($p < 0.05$). Nonetheless, a substantial difference was observed among the T2, T3, and T4 groups ($p < 0.05$). The Bursa Fabricius weight recorded the highest weight value in T4 as compared to the other groups, and the T1 group recorded the lowest weight value. Comparing the T1 group and the T4 group showed significant differences ($p < 0.05$).

Table 3. Combination effect of dietary *Bacillus licheniformis* and *Saccharomyces cerevisiae* on blood parameters in 35-day-old broiler chickens challenged with *E. coli*

Parameters	T1	T2	T3	T4
Electrolytes (µL)	1.60 ± 1.22^{a}	2.02 ± 0.45^{b}	1.73 ± 0.39^{c}	2.29 ± 0.54^{d}
Leukocytes (µL)	7.29 ± 2.62^{a}	8.58 ± 1.92^{b}	8.03 ± 1.20^{c}	6.03 ± 1.75^{d}
TPP (g/dL)	2.84 ± 0.36^{a}	2.4 ± 0.37^{b}	2.24 ± 0.17^{a}	2.60 ± 0.4^{c}
Fibrinogen (g/dL)	0.12 ± 0.18^{a}	0.04 ± 0.09^{b}	0.16 ± 0.17^{c}	0.2 ± 0.2^{d}
Hemoglobin (g/dL)	5.32 ± 1.72^{a}	5.32 ± 0.54^{b}	5.52 ± 0.91^{c}	6.13 ± 0.64^{d}
LDL (%)	59.04 ± 22.25^{a}	51.56 ± 5.51^{b}	53.02 ± 3.18^{c}	56.07 ± 9.32^{d}
HDL (%)	59.94 ± 4.67^{a}	47.08 ± 7.54^{b}	44.62 ± 7.80^{c}	50.87 ± 7.92^{d}
TG (%)	75.84 ± 5.52^{a}	80.86 ± 18.78^{b}	107.80 ± 68.45^{c}	70.27 ± 11.71^{d}
Cholesterol (%)	136.04 ± 28.30^{a}	117.50 ± 11.12^{b}	113.32 ± 12.57^{c}	128.03 ± 20.61^{d}

TTP: Total protein plasma; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides. Data are expressed as mean ± Standard deviation (SD). ^{a,b,c,d} Mean values with different superscript letters in the same row indicate significant differences ($P < 0.05$). T1: Control, T2: *Bacillus licheniformis* group, T3: *Saccharomyces cerevisiae* group, and T4: *Cerevisiae*.

Table 4. Combination effect of dietary supplementation of *Bacillus licheniformis* and *Saccharomyces cerevisiae* on total bacteria count of *E. coli* (log_{10}CFU) from the intestinal digesta

Treatment	Duodenum	Jejunum	Ileum	Cecum
T1	131.25 ± 34.28^{a}	210.25 ± 102.87^{a}	165.25 ± 30.57^{a}	276.50 ± 53.61^{a}
T2	66.25 ± 6.40^{ab}	81.00 ± 7.07^{b}	60.25 ± 4.27^{ab}	111.50 ± 28.52^{b}
T3	40.75 ± 21.85^{ac}	48.50 ± 12.48^{c}	83.75 ± 9.0^{ac}	79.00 ± 9.52^{ac}
T4	27.25 ± 4.11^{ad}	26.50 ± 6.14^{a}	49.75 ± 21.79^{ad}	71.50 ± 26.19^{ad}

Data are expressed as mean ± Standard deviation (SD). ^{a,b,c,d} Mean values with different superscript letters in the same column indicate significant differences ($p < 0.05$).

Table 5. Combination effect of dietary supplementation of *Bacillus licheniformis* and *Saccharomyces cerevisiae* on total bacteria count of *E. coli* (log_{10}CFU) from the intestinal digesta in 35-day-old broiler chickens challenged with *E. coli*

Treatment	Duodenum	Jejunum	Ileum	Cecum
T1	234.0 ± 92.76^{a}	186.0 ± 53.35^{a}	268.0 ± 121.82^{a}	277.25 ± 56.75^{a}
T2	62.75 ± 38.91^{ab}	169.5 ± 54.19^{b}	114.50 ± 32.52^{ab}	139.25 ± 128.49^{b}
T3	71.40 ± 28.74^{ac}	110.4 ± 57.29^{c}	70.2 ± 68.24^{ac}	90.20 ± 101.137^{ac}
T4	68.50 ± 31.44^{ad}	68.25 ± 69.95^{a}	40.5 ± 11.21^{ad}	99.25 ± 101.10^{ad}

Data are expressed as mean ± Standard deviation (SD). ^{a,b,c,d} Mean values with different superscript letters in the same column indicate significant differences ($p < 0.05$). T1: Control, T2: *Bacillus licheniformis* group, T3: *Saccharomyces cerevisiae* group, and T4: Combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*.

Table 6. Combination effect of dietary *Bacillus licheniformis* and *Saccharomyces cerevisiae* on carcass and immune organs weights in 35-day-old broiler chickens

	T1	T2	T3	T4
Carcass part				
Breast (g)	326.92 ± 17.67^{a}	356.16 ± 15.95^{b}	335.664 ± 7.98^{c}	392.0 ± 43.18^{ac}
Wing (g)	114.62 ± 10.37^{a}	120.86 ± 20.88^{b}	116.58 ± 7.72^{c}	113.52 ± 18.80^{d}
Thigh (g)	383.02 ± 48.13^{a}	378.13 ± 0.04^{b}	384.42 ± 57.45^{c}	316.06 ± 103.06^{d}
Immune Organs				
Spleen (g)	1.90 ± 0.66^{a}	3.58 ± 0.51^{ab}	3.78 ± 0.95^{ac}	4.54 ± 0.56^{ad}
Bursa Fabricius (g)	2.56 ± 0.313^{ad}	3.6 ± 0.48^{bd}	3.12 ± 1.05^{cd}	5.56 ± 0.88^{d}
Intestine (g)	146.84 ± 23.10^{a}	149.40 ± 26.85^{b}	236.22 ± 31.41^{c}	208.84 ± 18.08^{d}

Data are expressed as mean ± Standard deviation (SD). ^{a,b,c,d} Mean values with different superscript letters in the same row indicate significant differences ($p < 0.05$). T1: Control, T2: *Bacillus licheniformis* group, T3: *Saccharomyces cerevisiae* group, and T4: Combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*.

Table 7. Combination effect of dietary *Bacillus licheniformis* and *Saccharomyces cerevisiae* on carcass and immune organs weights in 35-day-old broiler chicken challenged with *E. coli*

	T1	T2	T3	T4
Carcass part				
Breast (g)	428.38 ± 23.38^{a}	453.6 6 ± 17.54^{b}	466.94 ± 44.08^{c}	499.00 ± 41.19^{d}
Wing (g)	258.4 ± 59.72^{a}	334.40 ± 38.89^{ab}	329.92 ± 14.24^{ac}	354.28 ± 17.54^{ad}
Thigh (g)	487.06 ± 35.03^{a}	493.42 ± 35.03^{b}	495.52 ± 49.21^{c}	452.34 ± 57.45^{d}
Immune Organs				
Spleen (g)	5.94 ± 2.39^{a}	17.50 ± 5.35^{ab}	14.14 ± 2.15^{ac}	23.12 ± 2.49^{ab}
Bursa Fabricius (g)	2.180 ± 0.48^{a}	4.060 ± 0.53^{b}	5.00 ± 0.60^{bc}	6.08 ± 1.77^{cd}
Intestine (g)	184.64 ± 12.26^{a}	189.40 ± 62.93^{b}	228.22 ± 35.08^{c}	216.44 ± 23.71^{d}

Data are expressed as mean ± Standard deviation (SD). ^{a,b,c,d} Mean values with different superscript letters in the same row indicate significant differences ($p < 0.05$). T1: Control, T2: *Bacillus licheniformis* group, T3: *Saccharomyces cerevisiae*, and T4: Combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*.

DISCUSSION

Blood profile

Both probiotics and prebiotics are popular feed additives that are crucial for improving the nutrition and overall health of poultry. The combination of probiotics and prebiotics improves the effectiveness in enhancing physiological parameters, health, and productivity of poultry. A technique whereby the active substances, probiotics, and prebiotics cooperate to enhance the physiological state of broiler chickens. The method by which probiotics and prebiotics, the active ingredients, work in concert to improve the physiological condition of poultry. A study by Sunu et al. (2021) used the combination of garlic extract (prebiotic) and *Lactobacillu*s (probiotic) to improve the blood profile and antioxidant capacity of broilers. This is consistent with the present study, whereby the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* groups improved the blood profile. Blood is an indicator of poultry health because it is a vital component in regulating the physiology of the body of poultry (Tugiyanti et al., 2016).

The amount of red blood cells in the blood is closely correlated with hemoglobin levels. Hemoglobin, the protein within red blood cells, is responsible for oxygen transport. A decrease in hemoglobin levels (anemia) can significantly impact poultry health by reducing oxygen delivery to tissues, impairing growth, and increasing susceptibility to infections. Fibrinogen is a plasma protein that plays a crucial role in blood clotting. While not considered a primary antioxidant, it can indirectly influence oxidative stress pathways (Mañucat-Tan et al., 2021). In this investigation, hemoglobin and fibrinogen levels were lowest in the control group, whereas the combination group of *Bacillus licheniformis* and *Saccharomyces cerevisiae* obtained the greatest value and differed significantly from the control group, the *Bacillus licheniformis* group, and the *Saccharomyces cerevisiae* group. This finding suggested the possibility that the *Bacillus cerevisiae* and *Saccharomyces cerevisiae* groups could work in concert to raise hemoglobin and fibrinogen levels.

The leukocytes play an essential role in protecting the body against various pathogens by way of phagocytes and production. A high number of leukocytes influences the immunity of the host; this high blood cell count indicates increased production of white blood cells to fight against infection. According to Table 3, the *Bacillus licheniformis* and *Saccharomyces cerevisiae* groups had the highest number of leukocytes, while the combination group had the lowest number when compared to the control group. Lawrence-Azua et al. (2018) discovered that adding dietary *Saccharomyces cerevisiae* to broiler diets resulted in high leukocyte levels, which supports the high leukocyte levels in the *Saccharomyces* group in this study. The increase in lymphocyte concentration indicates the high immune status of broilers supplemented with *Saccharomyces cerevisiae* because the mannan-oligosaccharides and beta-glucan components of the yeast cell wall have been reported to modulate immunity (Teng et al., 2021; Bi et al., 2022; Osman et al., 2024). The leukocyte levels in the current study, which are slightly lower than the normal range, may be influenced by the high environmental temperature and relative humidity that were recorded during the study. This could have contributed to potential stress in Cobb 500 broiler chickens.

Dietary probiotics and prebiotics have an influence on the concentration of blood lipids. The results of this study indicate that the concentration of plasma triglycerides, total cholesterol, HDL, and LDL is significantly different in all groups, with the control group exhibiting a higher concentration. This suggests that the dietary supplementation of *Bacillus licheniformis* and *Saccharomyces cerevisiae* affects the concentrations of the aforementioned plasma blood lipids. The results obtained were analogous to those published by Khalil et al. (2021), who indicated that various feed additives, including prebiotics and synbiotic combinations, affected the concentration of many plasma blood metabolites, namely cholesterol, triglycerides, LDL, and HDL. In this investigation, the concentration of cholesterol was decreased by the *Saccharomyces cerevisiae* group, while the triglycerides levels were decreased by the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* group reduced the triglycerides levels. As reported by Kumar et al. (2019) and Ahmed (2015), dietary *Saccharomyces cerevisiae* is responsible for the reduction of cholesterol levels. This implies that *Saccharomyces cerevisiae* might be responsible for the blood of broilers by affecting absorption and metabolism. Furthermore, it would support the cellular during its development and aid in the binding of cholesterol to the cellular surface (Mollinedo, 2012). As a result of the digestion of dietary components and the assimilation of fatty acids, triglycerides are produced in the intestinal mucosa and liver. Triglycerides in the treatment groups and the control group differed significantly, which suggests lipid metabolism (Table 3). According to Regar et al. (2019), the triglyceride concentrations for this study fall within the normal ranges of 75.84 ± 5.52, 80.86 ± 18.78, 107.80 ± 68.45, and 70.27 ± 11.71.

Carcass traits and immune organs

In this study, the carcass characteristics were improved by the addition of the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* in the broiler diet (Tables 6 and 7), which might be related to the inhibition of colonization by intestinal pathogens, thereby improving the utilization of nutrients in the diet. Probiotics can thrive in the digestive system when they are accompanied by prebiotics, as the two exhibit a synergistic effect that allows them to withstand an anaerobic environment, which is characterized by low pH, temperature, and oxygen. Moreover, the mannan and beta-glucan constituents of the yeast cell wall are utilized in conjunction with probiotic bacteria; the prebiotic effect is evidenced by their capacity to promote the growth, metabolism, and/or advantageous functions of probiotics, which translates into favorable carcass characteristics, thereby augmenting the weight of the thigh, wings, and breast, as observed in this study (Bilal et al., 2023; Elghandour et al., 2024). Nevertheless, the findings of the current investigation contradict the effect of probiotics, prebiotics, and symbiotics on carcass features shown in the research conducted by Salehimanesh et al. (2016).

The findings indicated that the combined *Bacillus licheniformis* and *Saccharomyces cerevisiae* group's immune organ weight increased (Tables 6 and 7). This is in contrast to Salehimanesh et al. (2016), who found no increase in immune organ weight following synbiotic treatment added to broiler diets. According to the study's results (Tables 6 and 7), the group that received a combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* had higher weights for the Bursa Fabricius, intestine, and spleen than the control group, *Bacillus licheniformis* only, and *Saccharomyces cerevisiae* only. The Bursa Fabricius is a hematopoietic location where B-cell maturation and antibody production take place. A low Bursa Fabricius weight may result in fewer lymphocytes, which may decrease the number of antibodies that serve as an indicator of immunity (Yazdi et al., 2014).

***E. coli* bacterial count**

The present result emphasized that the combination *of Bacillus licheniformis and Saccharomyces cerevisiae* group reduced the *E. coli* population as compared to the control group (Tables 4 and 5). The study by Sunu et al. (2021) supports this, showing that a synbiotic combination of *Allium sativum* and *Lactobacillus acidophilus* reduced the number

of bacteria, such as Coliform, *E. coli*, and lactic acid bacteria. The decrease in pathogenic bacteria in the chicken intestine is achieved by enhancing the generation of short-chain fatty acids, which affect host function (Liu et al., 2021; Ali et al., 2022; Huang et al., 2023). The creation of short-chain fatty acids by probiotics and prebiotics can boost the immune response by inducing the production of cytokines in the host's immune cells. This aligns with the current investigation, which revealed that the *combination of Bacillus licheniformis and Saccharomyces cerevisiae* diminished the *E. coli* colony counts before and after the chickens were challenged with *E. coli* relative to the control group. It is thought that *Saccharomyces cerevisiae's* ability to increase *Bacillus licheniformis* in the gastrointestinal tract helps the digestive system's competitive exclusion of pathogens, in this case, *E. coli*. The microbiome study has contributed to the creation of awareness about microorganisms, which has evolved from disease-causing agents that should be avoided to a more critical perspective that incorporates a more comprehensive understanding of their beneficial functions (Cunningham et al., 2021).

CONCLUSION

The *Bacillus licheniformis* (5×x10 9 CFU per 2g), and *Saccharomyces cerevisiae* (1,.0 ×x10 10 CFU per 2g) as a dietary supplementation modulated a synergistic effect on the blood profile (Electrolytes, Leukocytes, TPP, Fibrinogen, LDL, HDL, TG, and Cholesterol), organ immunity (Spleen, Bursa Fabricius, and Intestine) and intestinal health (Duodenum, Jejunum, Ileum, Cecum) after chickens were challenged with *E. coli*. The highest results of the study were observed in the combination of *Bacillus licheniformis* and *Saccharomyces* group (administered at a level of 1g per 1000g of basal diet). Therefore, the supplementation of a combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae* can be recommended to boost health and immunity in poultry production. Future research should explore the antioxidant capacity and molecular study of these dietary supplements. It is also necessary to explore the different levels or increments of the dosage of the combination of *Bacillus licheniformis* and *Saccharomyces cerevisiae*.

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Authors' contributions

Elly Tugiyanti contributed to conceptualization and design, data acquisition, and original draft preparation. Rosidi conducted the analysis and interpretation of the data. Mariama Abdula was involved in the evaluation and editing of the manuscript. All authors verified the article's most recent edition before publication.

Availability of data and materials

The data to support this study's findings is available upon reasonable request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time. Additionally, all authors checked the originality of data and sentences via plagiarism checkers.

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