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Effects of *Saccharomyces boulardii* on Productivity, Egg Quality, Excreta Microbiology, and Ammonia Emissions in Indonesian Local Ducks

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ABSTRACT

The Cihateup duck is an Indonesian waterfowl with potential as an egg producer. However, the productivity remains suboptimal. Dietary supplementation with the yeast probiotic *Saccharomyces boulardii* (*S. boulardii*) has been reported to improve poultry productivity. The current study aimed to evaluate the effects of *S. boulardii* yeast probiotic supplementation in Cihateup duck feed on production performance, egg quality, the microbial population in excreta, and excreta ammonia level. A total of 60 female Cihateup ducks aged 71 weeks were randomly assigned to four treatment groups (three replicates per treatment, five ducks per replicate). The treatment consisted of a control (T0), which was a basal diet without *Saccharomyces boulardii*; basal diet plus 0.5 g/kg feed *S. boulardii* (T1), basal diet plus 1.0 g/kg feed *S. boulardii* (T2), and basal diet plus 1.5 g/kg feed *S. boulardii* (T3). The feed treatment lasted for 8 weeks. Production performance, including duck day production, feed conversion ratio (FCR), and total and average egg weight, was evaluated weekly. At the end of the experiment, the physical quality of eggs, fat content, cholesterol, and fatty acid profile (saturated and unsaturated fatty acids) of egg yolks were analyzed. The analysis of excreta microbes and excreta ammonia levels was conducted in the eighth week. *Saccharomyces boulardii* supplementation significantly improved duck day production, total egg weight, and FCR in the T3 group, although it did not significantly affect egg physical quality across all treatments. The egg yolk cholesterol was significantly lower in the T1 and T3 groups than in the other groups. The present study also found that supplementation with *S. boulardii* increased the egg yolk omega-6 fatty acids (linoleic acid, γ-linolenic acid, and arachidonic acid), especially in T2 and T3 treatments. Ammonia in duck excreta decreased significantly in the probiotic groups compared with the control group, with the lowest level in T3. The findings of the present study demonstrated that *S. boulardii* has the potential as a probiotic for enhancing laying performance, improving the functional quality of eggs, and reducing excreta ammonia levels of Cihateup ducks.

Keywords: Cihateup duck, Egg quality, Performance, Probiotic, *Saccharomyces boulardii*

INTRODUCTION

Optimising poultry productivity is a challenge and essential to address the increasing demand for poultry products on a global scale, including meat and eggs. One strategic approach that has been developed is the intervention in nutrient absorption in the digestive tract through the use of feed additives (Bidura et al., 2019). Feed additives are widely recognised as specific nutritive and non-nutritive ingredients added to poultry feed (Perera and Ravindran, 2025). Feed additives have several benefits, including improving the quality and efficiency of feed utilisation and increasing the quantity and quality of poultry products (Özsoy et al., 2018). Probiotics are a type of feed additive that has been extensively studied,

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particularly after several countries announced a ban on antibiotic growth promoters (AGPs; Qiu et al., 2024). Exploration of natural alternatives such as probiotics has gained interest due to their ability to support livestock productivity and health (Wickramasuriya et al., 2022). Probiotics may contain one or more strains of microorganisms and are administered to poultry via feed or drinking water, either as bacteria, yeast, or a consortium of both (Abd El-Hack et al., 2020). *Saccharomyces* spp. yeast has a rich biological content, including proteins, amino acids, vitamin B complex, mannan-oligosaccharides, important trace minerals, and several other growth-promoting factors (Ahiwe et al., 2021).

Yeast-based probiotics, *Saccharomyces cerevisiae* (*S. cerevisiae*) have attracted widespread attention in poultry nutrition science due to their ability to improve gut microbiota balance and immune modulation (Abd El-Hack et al., 2020; Pratama et al., 2026). Similarly, *S. cerevisiae* var. *boulardii* has been investigated for its role as a probiotic in broiler chickens (Qin et al., 2018; Nari et al., 2020; Vu et al., 2025) and laying hens (Miraç and Cannan, 2025). *Saccharomyces boulardii* (*S. boulardii*) is known to improve gut health, trigger innate immunity, and collaborate with gut-dwelling microorganisms to protect the intestinal mucosa when consumed orally. *Saccharomyces boulardii*, with its anti-inflammatory properties, is enriched with nutrients and modulates the host's immune response (Zoghi and Darani, 2025). According to Manafi et al. (2018), *S. boulardii* supplementation in broiler chicken feed has been reported to reduce feed conversion rates and decrease the population of pathogenic bacteria in the caecum. Cihateup duck is one of West Java's leading duck breeds, originating from Tasikmalaya, West Java. Like other local Indonesian ducks, the Cihateup duck is a descendant of the Indian Runner duck, known as a laying duck. Male Cihateup ducks are generally used for meat production, while female ducks are used for egg production. However, the productivity of the local duck as an egg-laying type has not yet reached its optimal level (Ismoyowati et al., 2022).

A strategy to improve production performance and egg quality can be carried out by manipulating the gastrointestinal microbiota through probiotic supplementation (Bidura et al., 2019). A probiotic approach is expected to improve nutrient absorption, suppress colonization of pathogenic microorganisms, and ultimately increase productivity. The current study aimed to evaluate the effects of *S. boulardii* supplementation on production performance, egg quality, excreta microbial population, and excreta ammonia level in Cihateup duck feed.

MATERIALS AND METHODS

Ethical approval

All protocols in the experimental activities comply with standard operating procedures and were approved by Politeknik Negeri Jember, Indonesia, the animals' Ethics Committee, with registration number (106/PL17.4/PG/2024).

Animals, feed, and study design

A total of 60 Cihateup female ducks aged 71 weeks, with an average body weight of 1,550 ± 65 g. Cihateup ducks were kept in open pens using an intensive rearing system. The pen dimensions were 1.5 × 1.2 m (length × width). The pen temperature ranged from 23 to 32°C during the experimental period. The pen floor system consisted of 5 cm of rice husk litter, and the lighting duration was set to a minimum of 16 hours. Feed was delivered using a point feeding system at 120 grams per duck per day, and drinking water was provided *ad libitum*. The Cihateup ducks were randomly assigned to four treatment groups, with each treatment group consisting of three replicates. The treatment consisted of a control (T0), which was basal diet without *S. boulardii*, basal diet plus 0.5 g/kg feed *S. boulardii* (T1), basal diet plus 1.0 g/kg feed *S. boulardii* (T2), and basal diet plus 1.5 g/kg feed *S. boulardii* (T3). Dietary supplementation was implemented over 8 weeks, and the composition of the experimental feed is shown in Table 1. Nutrient requirements refer to the standard requirements for laying ducks (Leeson and Summers, 2005). The mixed concentrate duck feed, with a minimum crude protein of 38%, was produced by PT New Hope Indonesia. The *S. boulardii* population used was 10^8 CFU/g.

Table 1. Ingredients and chemical analysis of basal diet over 8 weeks of the study

Ingredient (percentage of dry matter)	**Percentage**
*Mixed concentrate duck feed	85
Rice bran	10
Azolla meal	5
Total	100
Composition	Content
Dry matter (%)	90.11
Metabolizable energy (Kcal/Kg)	2,800
Crude protein (%)	18.32
Ether extract (%)	4.74
Crude fiber (%)	5.32
Total ash (%)	12.85
Calcium (%)	4.11
Total phosphorus (%)	0.48

Leeson and Summers (2005) analyzed in the Laboratory of the Feed Science and Technology Division, Faculty of Animal Science, IPB University, Indonesia. *Mix concentrate duck feed contains: Soybean meal, fish meal, meat and bone meal, corn protein meal, micro-minerals, and a premix (vitamins, amino acids, and trace minerals).

Data and sample collection

The Cihateup ducks were observed for 8 weeks. Eggs were collected daily, and egg production was calculated daily and weekly. The egg mass was obtained by weighing the total daily eggs in each treatment repetition and averaging to obtain the weight per egg. Feed consumption was recorded daily and accumulated weekly. The feed conversion ratio (FCR) was calculated weekly in the form of w/w, that is, feed consumed (kilograms) per egg mass (kilograms). The production performance data were displayed and evaluated weekly until week 8. Physical quality tests of eggs, analysis of fat content, cholesterol, and fatty acid profile of egg yolks, analysis of excreta microbes, and analysis of excreta ammonia levels were performed in week 8.

Analysis of egg physical quality

The evaluation of egg physical quality includes both the exterior and interior parts of the egg. A total of 24 eggs for each treatment (eight eggs per replicate) were collected over four consecutive days during the eighth week of the study. Measurements and weighing were performed 24 hours after egg collection. Each egg was weighed using an Ohaus CL compact digital scale (CL-501T, OHAUS, USA). The eggs were broken on a glass table, and the height of the albumen and yolk was measured using a tripod micrometre (Mitutoya, No. 2050-08, 0.01-20 mm; Kawasaki, Japan). The length and width of the albumen and the diameter of the yolk were measured using digital callipers. The albumin and yolk indices, Haugh Unit (HU), for each replicate were calculated using the following formulas (Formula 1-3).

Albumin index = (albumen height/average of albumen length and albumen width) ×100 (Formula 1)

Yolk index = (yolk height/yolk diameter) × 100 (Formula 2)

Haugh units = [100 × log(H + 7.57 - 1.7W0.37)], where H is albumen height, and W is egg weight (Formula 3)

The eggshells were cleaned of any remaining albumin, separated from the egg membrane, and weighed. The thickness of the shell was measured at three areas of the egg (blunt end, pointed end, and middle) using a Mitutoyo Thickness Gauge 7301A 10/0.01 mm, Japan. The egg albumin and yolk are separated using an egg plastic separator, and the surface of the yolk is cleaned of any remaining albumin and chalaza. The yolk was weighed directly, and the egg white weight was calculated from the difference. The weight of the egg components is presented as a percentage. The yolk color was measured using the Roche yolk color fan scores (RYCF; F. Hoffmann-La Roche, Switzerland), which assigned ratings based on 15 distinct color samples, with 1 representing the lightest shade and 15 the darkest.

Fat content, fatty acids, and cholesterol of egg yolk

Six egg yolk samples per replicate were prepared and homogenized at low temperatures (10^{o}C). Fat content was analysed using AOAC Official Method 989.05, modified with the Mojonnier ether extraction method (1992), whilst the fatty acid profile was analysed in accordance with AOAC Official Method 969.33 (1997). The cholesterol content of egg yolk was determined using the principle of KOH saponification with alcohol, n-hexane extraction, and derivatization with trimethylchlorosilane (TMCS). The results were analyzed using gas chromatography–flame ionization detection (GC–FID) in accordance with method 18-6-5/MU/SMM-SIG (GC–FID) of PT SIG (Saraswanti Indo Genetech). Fat content and fatty acid profile are expressed as a percentage w/w, and cholesterol in mg/grams of egg yolk.

Excreta microbial population

Ten grams of excreta from each replicate were collected in sterile tubes for microbial population enumeration. One gram per sample was diluted into 9 mL of sterile phosphate-buffered saline (PBS) and diluted in a series from 10^{-1} to 10^{-7}. Subsequently, 0.1 mL of the last three dilution suspensions was inoculated onto the appropriate selective medium using the spread plate method. Lactic acid bacteria (Lactobacilli) were counted using dilutions of 10^{-5} to 10^{-7}, while *Salmonella* and *Escherichia coli* were counted using dilutions of 10^{-3} to 10^{-5}. Lactobacilli were cultured on MRS (de Man, Rogosa, and Sharpe) agar medium (Merck, Darmstadt, Germany) supplemented with 0.5% $CaCO_3$ (Telmadarre et al., 2020). Mac Conkey Agar media (Merck, Darmstadt, Germany) for *Salmonella* bacteria and Eosin Methylene Blue Agar (EMBA) medium for *Escherichia coli* (*E.coli*). The entire plate was incubated for 24-48 hours at 37°C. The number of colonies was counted with criteria of 25- 250 colonies per plate, and the result was multiplied by the dilution factor. Population data were expressed as logarithms (log colony-forming units per gram or log CFU per gram).

NH_3 (ammonia) excreta

The ammonia concentration in the excreta was determined using the Conway diffusion method, as described by Adjis et al. (2022). The test began by coating the rim of the Conway dish with Vaseline before use; subsequently, 1 mL of boric acid (H_3BO_3) with 2 drops of methyl red indicator was placed in the centre of the Conway dish. A total of 2.5 g of fresh duck excreta was mixed with 25 mL of distilled water, then homogenised, filtered, and centrifuged at 100 rpm for 3 minutes. Subsequently, 1 mL of the supernatant was collected and transferred to the left side of the dish, while 1 mL of saturated sodium hydroxide (NaOH) was placed on the right side. The Conway dish was then tightly sealed and gently shaken until the supernatant and NaOH were mixed. The dish was then left to stand at room temperature for 24 hours to allow NH_3 to bind to boric acid. The presence of bound ammonia is indicated by a color change in the boric acid indicator to green. After 24 hours, the boric acid indicator was titrated with 0.0057 N sulfuric acid (H_2SO_4) until the color changed from green to red. The concentration of NH_3 was calculated using the Formula 4 published by Adjis et al. (2022).

$$NH_3\,(mMol) = \frac{\text{Volume } H_2SO_4 \times \text{N } H_2SO_4 \times 1000}{\text{Sample volume (mL)}}$$

(Formula 4)

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) within a completely randomized design, with dietary yeast dose as the treatment variable. The statistical model employed was $Yij = \mu + Ti + \varepsilon ijY$, where Yij denotes the observed value, μ denotes the overall mean, Ti denotes the treatment effect, and εij denotes the experimental error. All statistical analyses were conducted using Minitab software (version 19). The normality of residuals and the homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively. If a significant treatment effect was found ($p < 0.05$), Tukey's honestly significant difference (HSD) test was used for pairwise comparisons of means.

RESULTS

Production performance

The effect of supplementing Cihateup duck feed with the yeast probiotic *S. boulardii* over an 8-week experimental period on production performance was evaluated and presented as averages over the experimental period (Table 2). Feed intake values showed no significant difference among replicates, as feed was provided under a controlled point feeding system with a fixed daily allowance of 120 g/duck/day, which was fully consumed at every feeding ($p > 0.05$). Duck day production (DDP), total egg weight, and feed conversion ratio (FCR) were significantly ($p < 0.05$) influenced by *S. boulardii* supplementation. The best performance was demonstrated by the T3 treatment group (1.5 g per kg feed), which showed the highest DDP percentage and total egg weight, as well as the lowest feed conversion ratio, compared to the control, T1, and T2 groups. The increases in DDP and total egg weight in the T3 group were 16.5% and 19.5%, respectively, compared to the control (T0). Meanwhile, the FCR (g/g) for T3 decreased by 17.45% compared to the control. Overall, treatment T3 consistently yielded the best results for most production performance variables (duck day, number of eggs, total egg weight, and FCR), while the average egg weight was not affected by the treatment. Treatments T0, T1, and T2 generally showed no significant differences ($p > 0.05$) among them.

Egg quality

Supplementation with *S. boulardii* for 8 weeks had no significant effect on average egg weight, yolk index, albumin index, yolk color, shell thickness, or the proportion of egg component weights ($p > 0.05$; Table 3). Haugh units, which indicate egg freshness, also showed no significant difference ($p > 0.05$) among all treatment groups. The present results indicated that the treatment did not alter the external and internal morphological structure of the eggs, nor did it affect the integrity of the shell or the pigmentation of the yolk in Cihateup ducks.

Fat content, fatty acids, and cholesterol of egg yolk

No significant differences were observed in the total fat content of Cihateup duck egg yolk across all groups ($p > 0.05$; Table 4). However, the mean fat content for group T3 was the highest among the treatments. The cholesterol content of egg yolk was significantly lower in treatments T1 (1,324.24 mg/100 g) and T3 (1,240.65 mg/100 g) compared with the control group (1,459.29 mg/100 g; $p < 0.05$). In terms of fatty acid profile, the percentage of oleic acid was the highest compared to other fatty acids in all treatment groups, followed by palmitic acid, stearic acid, and linoleic acid. The percentage of the saturated fatty acid stearic acid was significantly higher in T2 than in the control group. The percentage of the monounsaturated fatty acid oleic acid significantly differed ($p < 0.05$) between the T2 and T3 groups compared to the T0 and T1

groups. The highest percentage of oleic acid was reached by the T3 group, as the dose of *S. boulardii* increased. Similarly, the percentage of polyunsaturated fatty acids, which also belong to the omega-6 fatty acid group, namely linoleic acid, γ-linolenic acid, and arachidonic acid was significantly higher in T2 and T3 compared to the control ($p < 0.05$). In contrast to omega-6 fatty acids, the percentage of omega-3 fatty acid linolenic acid did not differ significantly ($p > 0.05$) across treatments, ranging from 0.23% to 0.41%. The ratios of PUFAs to SFAs and of ALA to LA in duck egg yolks were not statistically significant ($p > 0.05$) in all supplemented groups compared to the control. However, the mean values indicated a tendency towards an increase in these ratios in treatments T2 and T3. Overall, based on the study results, the percentage of total fatty acids in Cihateup duck egg yolks increased with increasing yeast probiotic supplementation dose.

Excreta microbial population

Microbiological analysis of duck excreta (Table 5) showed that the supplementation of *S. boulardii* probiotics in the diet did not affect total lactic acid bacteria, *Salmonella*, and *E. coli* in the excreta of ducks ($p > 0.05$) among the control and treatment groups. Similarly, the ratios of LAB/*E. coli* and LAB/*Salmonella* did not differ significantly ($p > 0.05$), although there was a tendency for both LAB/*E. coli* and LAB/*Salmonella* ratios increased in the yeast-supplemented groups (T1, T2, and T3) compared to the control.

NH3 (ammonia) excreta

Feed supplementation with the probiotic *S. boulardii* had a significant effect on the ammonia content of excreta ($p < 0.05$; Table 6). Ammonia in duck excreta decreased significantly ($p < 0.05$) across all experimental groups, as the dose of *S. boulardii* probiotic supplementation increased. The ammonia level in the T3 treatment group was the lowest among the treatments (122.22 mM/g). The percentage reductions in excreta ammonia from T1 to T3 relative to the control were 3.5%, 7.6%, and 16.8%, respectively.

Table 2. Production performance of laying ducks (71-79 weeks of age) during the 8-weeks of the study

Variables	T0	T1	T2	T3
Feed intake (g/wee)	840 ± 0	840 ± 0	840 ± 0	840 ± 0
Duck day (%)	67.48 ± 4.03[a]	68.44 ± 6.14[a]	63.26 ± 2.83[a]	78.64 ± 2.95[b]
Number of eggs (Eggs/week)	23.62 ± 1.41[a]	23.95 ± 2.15[a]	21.67 ± 1.72[a]	27.52 ± 1.03[b]
Total egg weight (g/week)	1,562.76 ± 133.58[a]	1,611.36 ± 179.34[a]	1,472.21 ± 108.34[a]	1,867.33 ± 78.74[b]
Average egg weight (g/egg)	66.14 ± 1.64	67.25 ± 3.48	67.88 ± 2.63	67.94 ± 0.62
FCR (g/egg)	181.65 ± 13.31[b]	179.61 ± 16.70[b]	201.04 ± 12.29[b]	154.24 ± 6.47[a]
FCR (g/g)	2.75 ± 0.27[b]	2.68 ± 0.30[ab]	2.97 ± 0.20[b]	2.27 ± 0.11[a]

[a,b] Means within a row with different superscripts differ significantly ($p < 0.05$). T0: Basal feed (containing 5% Azzola) without probiotics, T1: Basal feed plus probiotics 0.5 g/Kg, T2: Basal feed plus probiotics 1 g/Kg, T3: Basal feed plus probiotics 1.5 g/Kg, FCR: Feed conversion ratio

Table 3. Physical quality of laying ducks' eggs (79 weeks of age) after 8 weeks of the study

Variables	T0	T1	T2	T3
Egg weight (g)	68.15 ± 3.15	64.05 ± 3.48	65.48 ± 3.73	66.58 ± 3.54
Yolk weight percentage (%)	32.32 ± 3.27	32.13 ± 2.25	31.93 ± 3.61	32.13 ± 2.34
Albumen weight percentage (%)	56.77 ± 3.47	56.57 ± 2.84	57.00 ± 3.34	56.08 ± 2.23
Shell weight percentage (%)	11.00 ± 0.53	11.30 ± 0.77	11.07 ± 0.36	11.78 ± 0.90
Yolk index	43.81 ± 4.25	45.22 ± 2.61	43.21 ± 2.05	43.29 ± 7.50
Albumin index	12.48 ± 2.09	13.41 ± 1.90	13.44 ± 1.43	13.11 ± 1.55
Haugh unit	89.31 ± 3.71	91.74 ± 3.48	92.53 ± 2.72	93.08 ±3.87
Shell thickness (mm)	0.33 ± 0.02	0.32 ± 0.02	0.32 ± 0.03	0.34 ± 0.02
Yolk color	9.00 ± 0.89	8.83 ± 1.33	9.00 ± 2.00	8.33 ± 0.52

No significant differences were observed ($p > 0.05$). T0: Basal feed (containing 5% Azzola) without probiotics, T1: Basal feed plus probiotics 0.5 g/Kg, T2: Basal feed plus probiotics 1 g/Kg, T3: Basal feed plus probiotics 1.5 g/K

Table 4. Fat, cholesterol, and fatty acid profile of egg yolks in laying ducks (79 weeks of age) after 8 weeks of the study

Variables	T0	T1	T2	T3
Total fat (%)	4.96 ± 2.83	3.62 ± 1.45	7.54 ± 3.06	9.26 ± 3.06
Cholesterol (mg/g)	14.59 ± 0.85^{b}	13.25± 0.43^{a}	15.58± 0.51^{b}	12.41 ± 1.8^{a}
Saturated fatty acid (SFA)				
Butyric acid (%)	0.08 ± 0.02	0.07 ± 0.01	0.09 ± 0.03	0.12 ± 0.05
Myristic acid (%)	0.24 ± 0.05	0.34 ± 0.11	0.32 ± 0.02	0.35 ± 0.07
Palmitic acid (%)	21.05 ± 1.90	24.35 ± 2.69	26.90 ± 1.74	27.73 ± 0.75
Heptadecanoic acid (%)	0.15 ± 0.01	0.12 ± 0.05	0.13 ± 0.01	0.15 ± 0.06
Stearic acid (%)	4.15 ± 0.22^{a}	4.27 ± 0.59^{a}	5.28 ± 0.12^{b}	5.10 ± 0.28^{b}
Mono unsaturated fatty acid (MUFA)				
Palmitoleic acid (%)	1.54 ± 0.18^{a}	1.70 ± 0.28^{ab}	2.02 ± 0.21^{bc}	2.17 ± 0.23^{c}
Cis-10-heptadecanioc acid (%)	0.07 ± 0.03	0.07 ± 0.01	0.08 ± 0.02	0.09 ± 0.00
Elaidic acid (%)	0.19 ± 0.05	0.24 ± 0.12	0.20 ± 0.03	0.22 ± 0.04
Oleic acid (%)	35.87 ± 2.07^{a}	36.00 ± 4.51^{a}	44.15 ± 1.42^{b}	44.55 ± 1.95^{b}
Poly unsaturated fatty acid (PUFA)				
Linoleic acid (%)	3.47 ± 0.92^{a}	2.74 ± 0.20^{a}	6.48 ± 0.86^{b}	6.79 ± 0.98^{b}
y-Linolenic acid (%)	0.07 ± 0.03^{bc}	0.04 ± 0.01^{a}	0.12 ± 0.05^{c}	0.08 ± 0.02^{bc}
Linolenic acid (%)	0.41 ± 0.32	0.23 ± 0.05	0.39 ± 0.09	0.36 ± 0.14
Arachidonic acid (%)	0.55 ± 0.17^{a}	0.33 ± 0.06^{a}	1.88 ± 0.61^{b}	1.50 ± 0.52^{b}
Total SFA identified (%)	25.67 ± 1.99	29.15 ± 3.28	32.73 ± 1.63	33.46 ± 1.10
Total UFA identified (%)	42.17 ± 2.95	41.34 ± 4.58	55.33 ± 2.69	55.76 ± 3.46
Total MUFA identified (%)	37.67 ± 2.22	38.00 ± 4.67	46.45 ± 1.65	47.03 ± 2.13
Total PUFA identified (%)	4.50 ± 0.74	3.34 ± 0.21	8.87 ± 1.56	8.73 ± 1.45
PUFA:SFA	0.17 ± 0.02	0.12 ± 0.02	0.27 ± 0.05	0.26 ± 0.04
ALA: LA	18.07± 0.51	14.21± 4.60	18.08 ± 1.94	20.38 ± 5.95
Total fatty acids identified (%)	67.86 ± 4.95	70.49 ± 7.83	88.06 ± 3.77	89.23 ± 4.17

^{a,b,c} Means within a row with different superscripts differ significantly ($p < 0.05$). T0: Basal feed (containing 5% Azzola) without probiotics, T1: Basal feed plus probiotics 0.5 g/Kg, T2: Basal feed plus probiotics 1 g/Kg, T3: Basal feed plus probiotics 1.5 g/Kg; ALA: α-linolenic acid, LA: Linoleic acid

Table 5. Bacterial populations (log10 CFU/g) of the laying duck (79 weeks of age) excreta

Treatment	*Escherichia coli*	*Salmonella*	LAB	Ratio LAB/ *Eschericia coli*	Ratio LAB/ *Salmonella*
T0	5.06 ± 0.87	4.88 ± 0.62	6.23 ± 0.25	1.29 ± 0.21	1.34 ± 0.2
T1	4.10 ± 0.43	4.41 ± 0.86	6.55 ± 0.10	1.52 ± 0.97	1.41 ± 0.33
T2	4.93 ± 0.26	4.62 ± 0.71	6.7 ± 0.06	1.36 ± 0.68	1.45 ± 0.22
T3	4.77 ± 0.57	4.35 ± 0.077	6.74 ± 0.21	1.41± 0.22	1.55 ± 0.06

No significant differences were observed ($p > 0.05$). T0: Basal feed (containing 5% Azzola) without probiotics, T1: Basal feed plus probiotics 0.5 g/Kg, T2: Basal feed plus probiotics 1 g/Kg, T3: Basal feed plus probiotics 1.5 g/Kg. LAB: Lactic acid bacteria

Table 6. Ammonia (NH_3) content of the laying duck (79 weeks of age) excreta

Treatment	NH_3 (mM)
T0	146.93 ± 1.52^{d}
T1	141.77 ± 1.71^{c}
T2	135.79 ± 0.70^{b}
T3	122.22 ± 1.11^{a}

^{a,b,c,d} Means within a column with different superscripts differ significantly ($p < 0.05$). T0: Basal feed (containing 5% Azzola) without probiotics, T1: Basal feed plus probiotics 0.5 g/Kg, T2: Basal feed plus probiotics 1 g/Kg, T3: Basal feed plus probiotics 1.5 g/Kg

DISCUSSION

The supplementation of feed with yeast probiotics in laying ducks has not yet been extensively studied. However, the role of yeast-based probiotics has been extensively studied in other laying poultry, such as laying hens (Sarwar et al., 2022; Wahyuni et al., 2023; Qiu et al., 2024) and laying quails (Yousif and Kloor, 2023).

According to Abd El-Ghany (2025), the yeast strains *Saccharomyces cerevisiae* (*S. cerevisiae*) and *S. boulardii* are the most frequently used probiotic cultures in poultry feed formulations. The suggested doses of yeast to be supplemented in poultry feed range from 10^8 to 10^{10} CFU, as noted by Maksimović et al. (2022). In the present study, the type and dose of yeast used were appropriate for acting as a probiotic and supporting optimal poultry productivity. The results of the current study indicated that the probiotic *S. boulardii* supplementation influenced egg production (number of eggs and duck-day production), total egg weight, and FCR. No cases of disease or mortality were observed in the ducks during the study. Qui et al. (2024) reported consistent findings that yeast-based probiotics (2 g per kg feed) can increase egg production and weight as well as reduce the feed conversion ratio in laying hens. Hassanein and Soliman (2010) noted that supplementing laying hens with 0.4% and 0.8% yeast culture for 9 weeks during the late phase (weeks 70-79) could improve egg production, feed conversion, and egg mass through optimised nutrient absorption, modulation of the gut microbiota, increased levels of Lactobacilli, and a reduction in pathogenic bacteria. Similarly, the effect of supplementing laying quails with 2.5% probiotic yeast for 14 weeks also showed an increase in quail day percentage and egg weight, as well as a reduction in FCR (Yousif and Kloor, 2023).

Yeast culture is abundant in peptides, amino acids, vitamins, oligosaccharides, organic acids, minerals, and other advantageous components that enhance poultry production by facilitating the digestion and absorption of nutrients, thus improving poultry production performance (Liu et al., 2021; Qiu et al., 2024; Hossain et al., 2025). Furthermore, the increase in egg production resulting from yeast-based probiotic supplementation is strongly associated with improvements in gut health, the poultry immune system, and increased production and activity of digestive enzymes (Zhang et al., 2020). The mechanism related to yeast probiotics as a feed additive was also reported by Sun et al. (2019), who noted that mixed yeast supplementation (*Saccharomyces cerevisiae* and *Kluyveromyces maxianus*) has been associated with improved nutrient (dry matter) digestibility. The presence of yeast as a probiotic in the digestive tract leads to competition with pathogens for attachment sites on the intestinal wall and for nutrients, thereby suppressing pathogenic bacteria and promoting the development of beneficial gut flora (Ogbuewu et al., 2019). The processes underlying yeast's mechanism of action, as described in the paragraph, are consistent with our findings, which demonstrate enhanced productivity (egg production and FCR) in ducks.

No significant differences were found in the weight of eggs, albumin, yolk, or eggshell percentage, as well as albumin and yolk weight index, which aligned with those reported in other studies (Özsoy et al., 2018; Sarwar et al., 2022; Yousif and Kloor, 2023). In the present study, the dietary inclusion of *S. boulardii* also did not affect eggshell thickness, Haugh units, or yolk colour score. In contrast to previous studies, such as that by Hassanabadi et al. (2025), supplementation with 2 g of yeast extract per kg of feed significantly increased eggshell thickness in laying hens compared to the control (0.387 versus 0.362 mm). Findings by Park et al. (2020) also showed that the addition of 3% brewer's yeast hydrolysate per kg of layer hens' feed improved albumen height, Haugh unit, yolk colour, and eggshell thickness. Significant differences in the effects of yeast on layer poultry feed may be due to variations in the type of yeast used, dosage, duration of administration, age, and type of layer poultry used, as well as husbandry management conditions. Based on the Haugh unit calculations in the present study, the physical quality of duck eggs in all treatments can be categorised as 'excellent quality or AA grade' (over 72) according to United States Department of Agriculture (USDA) egg quality standards.

The fat content of duck eggs was not affected by supplementation of *S. boulardii* yeast, although the highest average fat content (9.26 ± 3.06) was found in the T3 treatment group (1.5 g per kg of feed). The results of the current study are consistent with the study by Qiu et al. (2024), which found that the addition of 2 g/kg of yeast culture increased the fat content of chicken egg yolks (8.49 ± 0.52%). In contrast, the cholesterol content of Cihateup duck egg yolks in groups T1 and T3 decreased significantly compared with the control group. The lowest cholesterol content was found in group T3 (12.41 mg/g). Ismoyowati et al. (2022) reported that dietary supplementation in Tegal duck (Indonesian local duck) with a commercial probiotic (2% in feed) showed a yolk cholesterol content of 13.99%, whilst the cholesterol content of the yolks of local Indonesian duck eggs reached 21.94 mg/g with 2% phenolic herbal supplementation in feed (Anggraeni et al., 2023). A reduction in egg cholesterol level following yeast probiotic supplementation was also reported by Yalçın et al. (2010), who reported that the addition of 0-4 g/kg of yeast to the diet of laying hens significantly reduced yolk cholesterol after 16 weeks of treatment. Similarly, Yalçın et al. (2014) reported that supplementation of yeast cell wall over a 26-

week study period significantly reduced yolk cholesterol, with a 13.35% reduction compared to the control. The finding of low cholesterol content in egg yolks (T3) in the current study is thought to be related to the β-glucan content in yeast, which is capable of binding cholesterol in the intestinal lumen, reducing lipoprotein absorption, and enhancing lipid metabolism in the liver (Aoe et al., 2020; Yu et al., 2021).

Another possible factor contributing to the reduction in yolk cholesterol levels is reduced absorption, synthesis, or both of cholesterol in the gastrointestinal tract, as explained in Xiao et al. (2012). Mannan-oligosaccharides (MOS) in the yeast cell wall play an important role in the cholesterol reduction effect. Mannan-oligosaccharides and β-glucans fermented by gut bacteria produce short-chain fatty acids (SCFAs), which acidify the large intestine and serve as a substrate for enterocyte energy production (Liu et al., 2021), thereby enhancing intestinal absorption and indirectly supporting lipid metabolism and reduced cholesterol deposition in the yolk (Bortoluzzi et al., 2018; Perricone et al., 2022). However, the effect of yeast probiotic supplementation on yolk cholesterol has not always shown the same effects across studies. In a study by Xiang et al. (2018), the combination of *S. boulardii* and *Pediococcus acidilactici* as probiotics in laying hens did not have a significant effect on reducing yolk cholesterol. The insignificant reduction in yolk cholesterol may be due to the relatively short duration of administration 5 weeks and the lower dose of 0.05 g/kg of feed. Studies involving bacterial probiotic supplementation over a longer period showed a trend of decreasing yolk cholesterol as the hens aged. The observed findings suggested that the cholesterol-lowering effect may become more pronounced with longer probiotic administration (Ramasamy et al., 2008).

The content of stearic acid, an SFA, was significantly higher in T2 and T3 than in the other treatments. In the current study, the dietary inclusion of *S. boulardii* was able to increase the content of several important fatty acids in the MUFA group, namely oleic acid (C18:1n9c), and PUFAs, namely linoleic acid (C18:2n6c), γ-linolenic acid (C18:3n6), and arachidonic acid (C20:4n6), which is an omega-6 fatty acid. The findings indicated that probiotic yeast modulates lipid metabolism in a beneficial, functional manner. An increase in linoleic acid (C18:2n6c) in chicken egg yolk was also observed as a significant effect of *Saccharomyces cerevisiae* supplementation at dosages of 0.05% and 0.1% of the diet (Özsoy et al., 2018). Yalçın et al. (2012) reported similar findings, noting that supplementation with 2 g of *Saccharomyces cerevisiae* per kg of feed increased oleic acid (C18:1n9c) levels in chicken egg yolk. The response of other (commercial) probiotic types to the increase in C18:2n6c and C18:3n3 fatty acids in duck eggs was also reported by Ismoyowati et al. (2022). However, in the present study, the increase in C18:2n6c and C18:3n3 was not accompanied by a significant change in the LA: ALA ratio across all treatments. The LA: ALA ratio (14.21-20.38) was higher than that reported by Özsoy et al. (2018), which ranged from 12.50 to 13.16. Meanwhile, in the same type of poultry, namely ducks, the LA: ALA ratio in our study was lower than that reported by Ismoyowati et al. (2022). The result indicates that probiotic supplementation in feed can elicit different fatty acid responses, which depend on the probiotic and the laying poultry used.

Yeast dietary supplements for chickens and ducks exert a modulatory effect on the intestinal microbiota, thereby enhancing colonization resistance against enteric pathogens (Pang et al., 2022). Furthermore, the role of *S. boulardii* in poultry is primarily reflected in enhancing the immune recognition and immune regulation functions of poultry, reducing the colonization of intestinal pathogens, and reducing the number of pathogenic bacteria in feces (Rajput et al., 2013). In the current study, the total counts of lactic acid bacteria, *Salmonella*, and *E. coli* in the excreta did not differ significantly among all treatments compared to the control. The number of *E. coli* bacteria (log10) tended to decrease in group T1, and the LAB/*E. coli* ratio tended to increase compared with the control. The LAB/*Salmonella* ratio was relatively higher in the T3 group. Wahyuni et al. (2023) reported that the addition of a combination of *Saccharomyces cerevisiae* (0.2%) and *Spirulina platensis* algae (0.3%) significantly increased lactic acid bacteria colonies and reduced coliform bacteria in the caecum of laying hens. A study by Pantaya et al. (2026) found that *Saccharomyces cerevisiae* markedly reduced intestinal populations of pathogenic bacteria, including *Salmonella*, *Shigella*, and *E. coli*, at a dose of 1.5 g per kg of feed. The ability of yeast to suppress pathogen populations is linked to several theories, including the notion that yeast cell wall β-glucans promote the growth of beneficial gut bacteria, such as *Lactobacillus* spp., thereby contributing to intestinal health (Fathima et al., 2023). Mannan-oligosaccharides, a component of yeast cell walls, can bind pathogenic bacteria, thereby preventing their adhesion to intestinal epithelial cells (Lee et al., 2021). An increase in the population of lactic acid bacteria, resulting from the presence of yeast in the digestive tract, indirectly initiates a mechanism of

competitive exclusion between gut pathogens, lactic acid bacteria, and yeast (Yeu et al., 2024).

The ammonia content in excreta decreased significantly as the dose of *S. boulardii* supplementation increased. The results are consistent with those reported by Wahyuni et al. (2023), who found that a 0.2% yeast dose reduced excreta ammonia by 52.82%. In the current study, T3 levels decreased by 16.8% compared to the control. Similar results were reported by Pantaya et al. (2026), who found that real-time ammonia gas emissions in Pekin ducks fed the probiotic *S. cerevisiae* decreased significantly. Rezaee et al. (2019) stated that probiotics can inhibit the activity of urease and other proteolytic enzymes that catalyse the deamination of amino acids and the hydrolysis of urea in the large intestine, thereby reducing the production of ammonia as a metabolic end-product excreted from the poultry's body. The reduction in ammonia excretion is also associated with increased digestibility of crude protein in the feed and nitrogen retention (Park et al., 2016). The more protein that is digested, the less nitrogen is lost in the excreta. The level of ammonia in excreta is typically determined by the amount of nitrogen in the excreta (Jeong and Kim, 2014).

The findings in the present study demonstrated that *S. boulardii* has the potential to serve as a probiotic for laying ducks. The protein, vitamin, and mineral content of yeast is a key factor in its safe use in the poultry sector (Liu et al., 2021). Moreover, yeast has functional mechanisms to modulate the immune system, histamine release, toxin binding, and interactions with pathogenic cells and gut constituents, thereby improving poultry performance (Hatoum et al., 2012). The presence of *S. boulardii* in the digestive tract of ducks in the present study allows for antagonistic activity of microorganisms, such as competition for nutrients, pH changes in the medium as a result of growth-coupled ion exchange or organic acid production, and secretion of antibacterial proteins and protease compounds (Pais et al., 2020).

CONCLUSION

The dietary supplementation of *S. boulardii* increased egg production and total egg weight, reduced the feed conversion ratio, and lowered the ammonia content of excreta. The present study also found that yeast probiotic supplementation decreased yolk cholesterol and increased the omega-6 fatty acid content (linoleic acid, γ-linolenic acid, and arachidonic acid) in egg yolks. In general, the optimal dosage of *S. boulardii* in the feed of Cihateup ducks was 1.5 g per kg. The findings demonstrated that *S. boulardii* has the potential to serve as a probiotic, leading to improved laying performance and indicating enhanced quality of Cihateup duck eggs. A limitation of the present study is that an in-depth analysis regarding the biological mechanisms by which probiotics influence gut microbiota composition and lipid metabolism was not directly investigated. Further studies on gut microbiota composition are suggested to substantiate the explanation of the beneficial effects of the probiotic *S. boulardii* on the productivity and lipid metabolism of laying ducks.

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Authors' contributions

Gilang Ayuningtyas contributed to the conceptualization of the study, method development, and its implementation. Validated and processed the data and drafted the original manuscript. Also addressed reviewers' comments, revised the statistical analysis, and updated the manuscript. Dadik Pantaya was involved in the conceptualization of the study, validated the methodology, supervised the study process and data processing, and was responsible for funding acquisition, also addressed reviewers' comments. Tera Fit Rayani, Gayuh Syaikhullah, Fitriani Eka Puji Lestari, Anis Usfah Prastujati, and Dwi Ahmad Priyadi contributed as study collaborators, recorded and curated the data, and participated in the writing of the manuscript. Hariadi Subagja supervised and reviewed the manuscript and was responsible for funding acquisition. All authors have read and agreed to the substance written in the final version of the manuscript.

Availability of the data and materials

Data available upon reasonable request from the corresponding author.

Competing interests

The authors declare no conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. All authors also state

that the AI tool (Paperpal by Editage) was used solely for literature search, language refinement, editing, and corrections during the drafting and revision of the manuscript. The authors checked all content and take full responsibility for the accuracy, originality, and integrity of the manuscript's final content, from submission and the revision process until final publication.

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