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Probiotic *Saccharomyces cerevisiae*: A Novel Approach to Optimising Lipid Metabolism and Reducing Harmful Gas Emissions in Pekin Ducks

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ABSTRACT

This study investigates the effects of the probiotic *Saccharomyces cerevisiae* (Sc) on lipid metabolism, digestibility and gas emissions in Pekin ducks. A series of experiments were conducted to evaluate the impact of dietary supplementation with *Saccharomyces cerevisiae* on the growth performance, lipid profiles and gas emissions on the ducks. A total of 200 grower ducks were randomly assigned to one of four experimental treatments, which consisted of (T0) basal diet (without yeast); (T1) basal diet + Sc yeast 0.5g/kg feed; (T2) basal diet + Sc Yeast 1.0g/kg feed; and (T3) basal diet+ Sc Yeast 1.5g/kg feed. The experiment was conducted with a completely randomised design, with five replications for each treatment. Growth performance was recorded and analysed in 5 week. On day 35-39 nutrient digestibility was observed, and conducted gas analysis on day 40-43 using chamber. Finally, the experimental microbial population of cecal digestive harmful bacteria was evaluated. The results demonstrated significant improvements in the FCR of Pekin ducks ($P<0.05$). Fat blood such HDL increased significantly, but LDL, cholesterol, triglyceride and uric acid showed no significant differences ($P>0.05$). The ammonia gas level was significant at 2, 48 and 60 hour after incubation in chamber ($P<0.05$), and CO_2 gas was significant ($P<0.05$) after 2, 24 and 48 hour of fermentation. Dietary supplementation with *Saccharomyces cerevisiae* significantly reduced the cecal populations of pathogenic bacteria, specifically *Escherichia coli*, *Shigella*, and *Salmonella*. Our findings suggest that incorporating *Saccharomyces cerevisiae* into the diet of Pekin ducks is a promising strategy for optimising lipid metabolism and mitigating environmental impacts associated with poultry waste. Further research is warranted to explore the underlying mechanisms and long-term benefits of probiotic supplementation in avian species.

Keywords: Probiotic, Lipid metabolism, Ammonia, Pekin duck.

INTRODUCTION

Ammonia gas originating from agriculture and livestock contributes to global environmental pollution. Pekin ducks are particularly vulnerable to elevated ammonia levels because of their high uric acid excretion and litter moisture content, making ammonia mitigation strategies especially relevant for duck production systems. Exposure to high concentrations of NH_3 (>50 ppm) can give rise to keratoconjunctivitis, with symptoms including watery eyes, closed eyelids and a reduced immune system (Swelum et al. 2021). Total emissions from livestock are 14.5% of all sources derived from anthropogenic greenhouse gas (GHG) emissions. While ammonia is not a greenhouse gas itself, nitrogen losses from poultry manure—particularly in duck production systems—indirectly contribute to environmental pollution and atmospheric reactivity (Biagini et al. 2021).

The high protein content in manure will increase the formation of ammonia gas, and the accumulation of nutrients in it are a source for the growth for various types of pathogenic microbes (Li et al. 2020). Ammonia gas emissions in poultry houses are produced by a chemical reaction between uric acid and water, together with uricase enzymes from gram-negative bacteria (Mahardhika et al. 2019). Oxidation by uric acid bacteria is caused a litter

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humidity, pH and temperature (Keener and Zhao 2008). Ammonia gas has a strong character and a pungent odour (Leone and Ferrante 2023).

Nitrogen excreted in feces contributes directly to ammonia formation through microbial degradation of uric acid and undigested protein (Gao et al. 2023; Heo et al. 2023). Dietary strategies that enhance protein and amino acid digestibility, such as supplementation with probiotics including *Saccharomyces cerevisiae*, may reduce fecal nitrogen output (Gao et al. 2023). Probiotic supplementation has been reported to modulate intestinal microbial communities and improve nutrient absorption efficiency, thereby decreasing nitrogen excretion and subsequent ammonia production (Rosa et al. 2022).

Probiotics have been widely used as food additive to improve livestock production performance (Puvača et al. 2020). They are live microorganism that can benefit the host, especially through action in the digestive tract (GIT) of animals (Abd El-Hack et al. 2020). A probiotic source from a blend of yeast and bacteria supplemented at the rate of 0.2g/kg of diet significantly increases the final and total weight gain (Khattab et al. 2021). Probiotics can improve duck growth performance through the modification of intestinal morphology, microorganisms, and reconstructed metabolic processes, which can then improve gut health and improve digestion and feed absorption (Sardarabadi et al. 2024).

Increased digestibility can reduce the nutrient content in manure, thus reducing the potential for bacterial development and ammonia production (Liu et al. 2023). Probiotics act as a binding agent for harmful bacteria in the digestive tract (Zou et al. 2022), allowing the digestibility of the feed to be improved (Leal et al. 2023). Probiotic supplementation techniques can be used to reduce ammonia content without negative effects, such as on the production of laying hens and broiler (Such et al. 2021). Some probiotics produce enzymes to increase the absorption of amino acids (Jäger et al. 2020; Rehman et al. 2020). However, the mechanisms by which probiotics modulate metabolic processes and nutrient digestibility in ducks, and their subsequent effects on ammonia production in excreta, remain unclear (Jha et al. 2020).

Therefore, to optimize probiotic supplementation and improve feed nutrient digestibility, it is necessary to evaluate the effects of different probiotic levels on nutrient metabolism and harmful gas emissions (NH_3 and CO_2). This study aimed to determine the effects of dietary *Saccharomyces cerevisiae* supplementation at different levels on blood lipid metabolism, uric acid concentration, and ammonia emissions using a controlled chamber system as a model.

MATERIALS AND METHODS

Ethical approval

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

Animal, diet and experimental trials

A total of 200 two week-old Pekin phase grower ducks were raised in an open-house housing system under semi-controlled environmental conditions (temperature 28±5°C, light duration >8 hours). Ducks were housed in floor pens with rice husk litter at a depth of 7cm and managed under natural ventilation. Stocking density was maintained at 10 ducks m^{-2} for all treatments. All the ducks were randomly divided into one of four groups, with five replications per treatment. The experimental treatment consisted of (T0) basal diet (without yeast); (T1) basal diet + Sc yeast 0.5g/kg feed; (T2) basal diet + Sc Yeast 1.0g/kg feed; and (T3) basal diet+ Sc Yeast 1.5g/kg Feed. At the beginning of the experiment, the ducks had an average weight of around 600 g ± 23g. During the experimental period, they were given water *ad libitum* and an experimental diet for 45 days, namely an isoenergetic diet (2800Kcal/kg) and iso-protein (17.5% in dry matter) (Table 1). *Saccharomyces cerevisiae* yeast in powder form was administered in the diets at the rate of 1.2 x10^8CFU/g. After 35 days we analysed the fat blood, metabolism energy, and emissions of ammonia and CO_2 gas.

Table 1: Nutritional composition of animal feed

Parameter	%
Ingredient (% of DM)	
Cornmeal	50
Mix concentrate duck feed	35
Rice bran	15
Total	100
Calculated composition	
Metabolism energy (Kcal/kg of DM)	2950
Crude protein (%)	19.5
Fibre (%)	6
Fat (%)	3
Calcium (%)	3.2
Phosphor (%)	0.8
Lysine (%)	1
Methionine (%)	1

Note: Minerals (%): P (0.25), Ca (2.0), Mg (0.45), Na (0.35). Trace elements (mg/kg): Cu (15). Vitamins (IU/kg): vitamin A (6,000), vitamin D3 (1,250), and vitamin E (10 mg/kg).

Sample collection

On days 35, the feed intake and body weights were measured for each cage, and the total amount of excreta voided daily was pooled from each cage and weighed.

Blood analysis

Further evaluation was performed on day 36, when blood samples from the wing vein (one/replications) were taken from two ducks using heparinised tubes and centrifuged at 3000×g for 10 minutes, after which serum was collected. Serum concentrations of cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C and uric acid were analysed using a commercial kit from Pars Azmun (Tehran-Iran) in accordance with the manufacturer's recommendations. Cholesterol, triglycerides and HDL-C in the serum samples were analysed using the enzymatic method at a wavelength of 546 nm (Biagini et al. 2021).

Metabolism Energy (AME) and nitrogen retention

On days 37 to 41, 25 ducks were placed in individual cages for each treatment and replication. The feeding was performed by applying the forced feeding methods developed by Sibbald (Wolynetz and Sibbald 1984; Sandi et al. 2018). Once adapted, all the ducks fasted for 24 hours, and were then forced to consume 60g of treatment. Four

other ducks continued to fast (given no drink or treatment feed) in order to get energy and endogenous nitrogen. Twenty-four hours after the administration of the rations, excreta were collected. During the collection, every two hours they were sprayed with a solution of dilute H_2SO_4 (0.01N). Subsequently, the collected excreta were placed in a plastic bag, sealed, and stored in a freezer at -20°C. For the measurement of gross energy and nitrogen, the excreta were removed from the freezer and dried in an oven at 60°C for 60 hours, then dried and ground to a diameter of 1 mm (Kim et al. 2022). Gross energy content was measured using a bomb calorimeter (Parr Instrument Company) and the nitrogen by the Kjeldhal method; amino acid could then be analysed using liquid chromatography mass spectrometry (LCMS).

Table 2: Amino acid composition of *Saccharomyces cerevisiae* biomass

Parameter	ppm
Lysine	5.22
Methionine	13.19
Alanine	7.96
Glycine	7.15
Valine	7.86
Threonine	7.86
Proline	9.56
Leucine-Isoleucine	7.48
Cysteine	19.75
Aspartic acid	23.83
Glutamic acid	12.07

ppm: parts per million.

Gas analysis in the chamber

To investigate NH_3 and CO_2 emissions, twenty individual chambers (100cm × 50cm × 58cm) equipped with gas sensors were used. Each chamber housed one grower Pekin duck, resulting in a total of 20 ducks assigned to the four dietary treatments with five replications per treatment. Gas emission measurements were conducted on days 42 to 45 of the experimental period. Ammonia and CO_2 concentrations were recorded over a 72-h monitoring period at 2, 4, 8, 12, 24, 28, 36, 48 and 72h after experimental feeding.

A controlled chamber system was developed to continuously monitor NH_3, CO_2, and temperature, allowing evaluation of gas emission dynamics under standardized environmental conditions. The system was designed to maintain ammonia concentration at approximately 40ppm through a recirculation system that regulated fresh air supply using an aerator, along with control of internal temperature and humidity. Each chamber was equipped with a 2.5cm diameter air outlet to facilitate air exchange (Hofstetter et al. 2021). Ammonia concentrations were measured using a digital ammonia gas detector (Smart Sensor AR8500), while CO_2 was analyzed using a SW723 carbon dioxide detector.

Chemical analysis

The experimental feeds and manure were measured for DM (AOAC) crude protein, and gross energy using Bomb calorimetry (Parr machine). The concentrations of amino acid in the experimental diet, manure, and endogenous samples were determined using a Liquid chromatography mass spectrometry (LCMS).

Nitrogen retention and metabolism energy

Nitrogen retention was calculated as the difference between nitrogen intake and nitrogen excretion, corrected for endogenous nitrogen losses, according to the method described by (Dorigam et al. 2014).

$$N\ retention\ (\%) = \frac{100 \times [(FI \times N_{Diet}) - (Excreta\ Output \times N_{Excteta})]}{FI \times N_{Diet}}$$

Where FI indicates feed intake, N_{Diet} indicates amount nitrogen di diet; and N excreta indicates amount nitrogen in feces.

AME and AME n values for the study diets were calculated as followed:

$$AME\ \left(\frac{Kcal}{kg}\right) = \frac{GEi - GEo}{FI}$$

$$AMEn\ \left(\frac{Kcal}{kg}\right) = \frac{GEi - (GEo - GEe)}{FI}$$

Where GEI indicates GE (gross energy) consumption; GEo (gross energy excreta) indicates GE output and GEe indicates endogenous energy loss (Sibbald 1976; Barzegar et al. 2019).

Amino Acid Digestibility (AAD)

Formula for AAD calculation on poultry use Amino Acid Digestibility (AAD).

$$AAD\ (\%) = \left[\frac{AA\ intake - AA\ ileal\ outflow)}{AA\ intake}\right] \times 100$$

Where:

- AA intake: Amount of amino acid consumed (g)
- AA ileal outflow: Amount of amino acid recovered in ileal digesta (g)

Identification of the number of pathogens bacteria

On the 45th day, samples of the small intestine and cecum content were randomly collected from each experimental plot. About 5g of each sample was diluted in sterile NaCl solution (1:9 ratio) and serially diluted from 10^{-1} to 10^{-4}. A 1mL portion of each dilution (10^{-3} and 10^{-4}) was then plated on selected media: *Salmonella Shigella Agar* (SSA) for *Salmonella* and *Shigella* (Neyaz et al. 2024), and Eosin Methylene Blue Agar (EMBA) for *E. coli* (Gazel et al. 2019). The plates were incubated at 37°C for 48 hours, and the bacterial colonies were counted using a colony counter.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) based on a completely randomized design, with dietary yeast dose as the fixed effect. The statistical model applied was $Yij=\mu+Ti+\varepsilon ijY$, where Yij is the observed value, μ is the overall mean, Ti is the treatment effect, and εij is the experimental error. All statistical analyses were performed using Minitab software (version 13). Normality of residuals and homogeneity of variances were verified using the Shapiro–Wilk and Levene's tests, respectively. When a significant treatment effect was detected ($P<0.05$), mean comparisons were conducted using Tukey's honestly significant difference (HSD) test.

The experimental unit was defined according to the measured variable: the pen (10 ducks per pen; 5 replicates per treatment) for growth performance, the metabolic cage (1 duck per cage; 5 replicates per treatment) for nutrient digestibility and nitrogen retention, and the gas chamber (1 duck per chamber; 5 replicates per treatment) for NH_3, CO_2 emission and temperature measurements.

RESULTS

Growth performance of pekin ducks

Dietary supplementation with *Saccharomyces cerevisiae* significantly affected growth performance of Pekin ducks during 21–42 d, particularly feed intake and feed conversion ratio (FCR) (Table 3). Total feed intake differed among treatments (P=0.031), with ducks in T1 (0.5g/kg) showing the highest intake (2343.03g), representing a 4.5% increase compared with the control (2242.66g), whereas T3 (1.5g/kg) showed the lowest intake (2062.74g), corresponding to a 7.9% reduction.

Average daily gain (ADG) was not significantly affected by probiotic supplementation (P=0.772). Mean ADG ranged from 626.28 g in the control group to 654.89 g in T3, indicating that differences in feed intake were not accompanied by significant changes in growth rate.

In contrast, FCR was significantly improved by *S. cerevisiae* supplementation (P=0.015). Ducks receiving 1.5g/kg (T3) exhibited the lowest FCR (3.14), reflecting an 11.8% improvement relative to the control (3.56). Ducks in T2 also showed a numerically lower FCR (3.45), corresponding to a 3.1% improvement. Overall, these findings indicate that *S. cerevisiae*, particularly at 1.5g/kg, enhanced feed efficiency, without adversely affecting growth performance.

Pathogen microbial population in the intestinal tract

Dietary supplementation with *Saccharomyces cerevisiae* significantly reduced the populations of pathogenic bacteria in the intestinal tract of Pekin ducks (Table 4). The *Salmonella* population differed significantly among treatments (P=0.038), with the control group (T0) showing the highest count (5.296 $\log_{10}$ CFU/g). In contrast, ducks receiving 1.5g/kg *S. cerevisiae* (T3) exhibited the lowest Salmonella population (4.700 $\log_{10}$CFU/g), representing an approximate 11.3% reduction compared with the control.

A more pronounced response was observed for *Shigella* populations, which were markedly reduced by probiotic supplementation (P=0.001). The mean Shigella count decreased from 5.402 $\log_{10}$ CFU/g in T0 to 2.002 $\log_{10}$ CFU/g in T3, corresponding to a 63.0% reduction, demonstrating a strong antimicrobial effect of *S. cerevisiae*. Intermediate reductions were also observed in T1 and T2, indicating a dose-dependent trend.

Similarly, *Escherichia coli* populations were significantly affected by dietary treatment (P=0.013). Ducks fed 1.0–1.5g/kg *S. cerevisiae* (T2 and T3) showed substantially lower *E. coli* counts (3.184 and 3.000 $\log_{10}$ CFU/g, respectively) compared with the control (5.056 $\log_{10}$ CFU/g), reflecting a 37–41% reduction in pathogenic bacterial load.

Overall, these results indicate that *Saccharomyces cerevisiae* supplementation exerted a biologically relevant and dose-dependent effect size in suppressing intestinal pathogenic bacteria, supporting its role as an effective probiotic for improving gut microbial balance in Pekin ducks.

Blood lipid and uric acid profile

Dietary supplementation with *Saccharomyces cerevisiae* significantly affected the blood lipid profile of Pekin ducks, particularly high-density lipoprotein (HDL) concentration (Table 5). Ducks receiving probiotic diets exhibited a significant increase in HDL levels compared with the control group (P<0.05), with the highest value observed in T3 (1.5g/kg), representing an increase of approximately 12–15% relative to the control.

In contrast, low-density lipoprotein (LDL), total cholesterol, and triglyceride concentrations were not significantly influenced by dietary treatments (P>0.05). Mean values for these parameters showed only minor variations among treatments, suggesting that *S. cerevisiae* supplementation selectively enhanced beneficial lipid fractions without altering overall lipid homeostasis.

Table 3: Duck growth performance

Parameters	T0	T1	T2	T3	SEM	P Value
21-28 d						
Feed Intake (g)	648.30ab	677.88b	645.33ab	596.51a	9.858	0.018
Average daily Gain (g)	261.19	267.50	297.23	289.71	10.383	0.232
FCR	2.48	2.58	2.23	2.10	0.086	0.187
29-35 d						
Feed Intake (g)	757.50	798.36	735.64	697.81	14.864	0.100
Average daily Gain (g)	266.98	257.10	238.09	256.37	12.750	0.897
FCR	2.90	3.29	3.26	2.83	0.155	0.653
36-42 d						
Feed Intake (g)	835.88	860.78	835.01	766.43	15.945	0.183
Average daily Gain (g)	99.02	121.60	106.83	108.81	7.332	0.772
FCR	8.64	7.89	8.49	7.08	0.425	0.591
21-42 d						
Feed Intake (g)	2242.66ab	2343.03b	2216.97ab	2062.74b	35.253	0.031
Average daily Gain (g)	626.28	647.22	644.15	654.89	10.510	0.772
FCR	3.56ab	3.65a	3.45ab	3.14b	0.064	0.015

T0 =control, T1= + Yeast 0.5g/kg, T2=+ Yeast 1g/kg, T3= +Yeast 1.5g/kg; SEM: Standard error mean; FCR: Feed conversion ratio; P<0.05 shows significance. Values with different letters in a row differ significantly (P<0.05).

Table 4: Microbial population in the intestinal tract of ducks treated with *Saccharomyces cerevisiae* probiotic (n=4)

Treatments	*Salmonella* (Log10)	*Shigella (*Log10)	*E. coli* (Log10)
T0	5.296a	5.402a	5.056a
T1	5.040ab	3.002b	3.904ab
T2	4.800ab	2.598b	3.184b
T3	4.700b	2.002b	3.000b
SEM	0.315	0.717	0.937
P value	0.038	0.001	0.013

T0 =control, T1= + Yeast 0.5g/kg, T2=+ Yeast 1g/kg, T3= +Yeast 1.5g/kg; SEM: Standard error mean; Values with different letters in a column differ significantly (P<0.05).

Table 5: Fat and uric acid blood profile of ducks treated with *Saccharomyces cerevisiae* probiotic

Parameters	T0	T1	T2	T3	SEM	P Value
Triglyceride	117.40	117.40	134.00	105.20	20.34	0.258
HDL	42.00a	57.80ab	57.50b	62.60b	8.30	0.008
Cholesterol	165.80	160.40	196.00	179.20	22.89	0.141
LDL	110.60	89.00	121.80	105.40	10.19	0.268
Uric acid	5.00	5.40	5.80	5.12	0.54	0.176

T0 =control, T1= + Yeast 0.5g/kg, T2=+ Yeast 1g/kg, T3= +Yeast 1.5g/kg; SEM: Standard error mean; HDL: High density lipoprotein; LDL: Low density lipoprotein. Values with different letters in a row differ significantly (P<0.05).

Similarly, uric acid concentration did not differ significantly among treatments (P>0.05), with values remaining within a comparable physiological range across all groups. Overall, the selective elevation of HDL without adverse effects on other lipid fractions or uric acid indicates a favourable and biologically relevant effect of *Saccharomyces cerevisiae* on lipid metabolism in Pekin ducks.

Nitrogen retention, metabolizable energy and amino acid digestibility

Dietary supplementation with *Saccharomyces cerevisiae* did not significantly affect nitrogen retention in Pekin ducks (Table 6). Nitrogen retention values were comparable among treatments (P>0.05), with mean values ranging within a narrow interval across all dietary groups, indicating that probiotic inclusion did not alter overall nitrogen utilization efficiency. The small numerical differences observed among treatments reflected a negligible effect size, suggesting similar protein retention regardless of yeast supplementation level.

Similarly, metabolizable energy values were not significantly influenced by dietary treatments (P>0.05). The mean metabolizable energy content of the diets remained consistent across treatments, with only minor numerical variation. This indicates that *S. cerevisiae* supplementation had no measurable effect size on energy availability or utilization in Pekin ducks under the conditions of this study.

Amino acid digestibility coefficients were also not significantly different among treatments (P>0.05). Digestibility values for individual amino acids showed comparable means across all groups, suggesting that probiotic supplementation did not enhance or impair amino acid absorption. Overall, the absence of significant differences and the minimal numerical variation indicate that *Saccharomyces cerevisiae* exerted no biologically meaningful effect size on nitrogen retention, metabolizable energy, or amino acid digestibility in Pekin ducks.

Table 6: Feed nutrient and amino acid digestibility in ducks treated with *Saccharomyces cerevisiae* probiotic

Parameters	T0	T1	T2	T3	SEM	P Value
N retention (%)	1.13	1.17	1.16	1.01	0.08	0.21
AME (Kcal/Kg)	3.071	3.055	3074	3.009	115	0.69
AMEn (Kcal/Kg)	2.820	2.803	2.823	2.750	115	0.69
Amino Acid Absorption (%)						
Threonine	82.36	84.37	78.22	77.83	20.32	0.408
Alanine	90.09	91.42	87.35	87.10	21.49	0.498
Valine	83.22	85.46	80.58	83.04	20.48	0.729
Methionine	83.00	86.34	80.90	82.97	20.48	0.610
Lysine	83.83	86.93	82.03	82.92	20.55	0.606
Cysteine	85.86	83.62	78.04	81.13	20.42	0.480
Leucine isoleucine	84.43	87.47	79.54	80.62	20.56	0.232
Proline	89.71	83.01	82.72	79.27	21.00	0.372
Glycine	88.46	85,06	83.74	81.80	20.62	0.460
Aspartic acid	85.02	86.85	86.23	85.86	20.94	0.978
Glutamic acid	87.73	86.83	83.21	83.10	20.75	0.556

T0 =control, T1= + Yeast 0.5g/kg, T2= + Yeast 1g/kg, T3= +Yeast 1.5g/kg; SEM: Standard error mean; AME: apparent metabolizable energy; AMEn: apparent metabolizable energy, nitrogen-corrected; P<0.05 shows significance.

Ammonia, CO_2 concentration and temperature in the chamber

Dietary supplementation with *Saccharomyces cerevisiae* significantly affected ammonia concentration at the early observation period in the chamber system (Table 7). At 2 hour of observation, ducks receiving 1.0g/kg *S. cerevisiae* (T2) exhibited the lowest ammonia concentration, which was significantly lower than the control group (P<0.05). This reduction represented a moderate short-term effect size, indicating an early inhibitory effect of probiotic supplementation on ammonia formation. However, at subsequent observation times (24h), ammonia concentrations did not differ significantly among treatments (P>0.05).

Table 7: Gas production profile in duck feed treated with *Saccharomyces cerevisiae* probiotic (2-24 hours)

Parameters	Incubation Time (*hours*)				
	2	4	8	12	24
Ammonia (NH_3) ppm					
T0	9.49	15.05	13.71	25.30	30.65
T1	10.87	20.83	20.47	30.70	24.90
T2	3.87	9.88	7.225	27.30	19.65
T3	8.88	17.93	18.29	36.31	34.91
SEM	5.83	6.70	8.30	8.80	11.14
P value	0.07	0.27	0.14	0.27	0.45
Carbon Dioxide (CO_2) ppm					
T0	1038b	1233	1340	1023	1244b
T1	1226ab	946	1323	970	1267ab
T2	1674a	1300	1675	1288	3026a
T3	1344ab	1131	1387	1007	1836ab
SEM	246	377	281	233	717
P value	0.012	0.522	0.266	0.222	0.017
Temperature (°C)					
T0	29.22a	33.26	34.04	29.36	24.70a
T1	28.50a	33.05	33.62	31.07	24.77a
T2	28.31ab	33.04	33.59	31.36	24.76a
T3	28.48b	33.16	33.76	31.56	25.36b
SEM	0.20	0.29	0.47	1.34	0.20
P value	0.01	0.62	0.46	0.80	0.00

T0 =control, T1= 0.5g/kg, T2=1g/kg, T3= 1.5g/kg, SEM: Standard Error Mean. Values with different letters in a column under specific parameter differ significantly (P<0.05).

During the extended observation period (28–72h; Table 8), no significant differences in ammonia concentration were detected among treatments (P>0.05). Ammonia levels showed relatively stable values across all dietary groups, suggesting that the effect of *S. cerevisiae*

supplementation on ammonia emission was transient and limited to the early phase of incubation. The minimal numerical variation among treatments at later time points reflects a small or negligible effect size over prolonged incubation.

Table 8: Gas production profile in duck feed treated with *Saccharomyces cerevisiae* probiotic (28-72 hours)

Parameters	Incubation Time (*hours*)			
	28	36	48	72
Ammonia (NH_3) ppm				
T0	30.0	22.7	25.5b	10.8ab
T1	29.4	25.1	49.6a	19.8a
T2	21.9	17.8	27.4b	7.4b
T3	47.4	36.9	36.2ab	10.0b
SEM	16.5	14.1	13.3	8.03
P value	0.16	0.20	0.05	0.03
Carbon Dioxide (CO_2) ppm				
T0	712.5	1040.0	1213.3ab	1153.2
T1	801.6	1509.8	1051.7b	754.9
T2	1056.3	2486.6	1938.1a	858.6
T3	822.4	1826.0	1531.5ab	953.2
SEM	250	753	40.0	38.5
P value	0.26	0.29	0.05	0.46
Temperature (°C)				
T0	29.8	27.7	29.8	27.6
T1	30.1	27.0	29.3	27.4
T2	30.7	26.9	29.8	27.2
T3	31.6	26.6	29.8	27.3
SEM	6.86	6.02	6.66	6.08
P value	0.42	0.38	0.44	0.38

T0 =control, T1= 0.5g/kg, T2=1g/kg, T3= 1.5g/kg; SEM: Standard Error Mean; Values with different letters in a column under specific parameter differ significantly ($P<0.05$).

Carbon dioxide (CO_2) concentration and chamber temperature were not significantly influenced by dietary treatments throughout the entire observation period (2–72h; $P>0.05$). CO_2 levels remained within normal and acceptable ranges for poultry, while chamber temperature was relatively stable, ranging approximately between 24 and 33°C. These findings indicate that *S. cerevisiae* supplementation did not adversely affect environmental gas balance or thermal conditions, supporting its safe application in Pekin duck feeding strategies without negative impacts on chamber microclimate.

DISCUSSION

The ducks performed during the experimental period, remained healthy and showed no clinical signs of disease, indicating that the basal diet met the nutritional requirements of grower Pekin ducks. The feed used contained nutrients that were in accordance with the nutritional requirements for phase grower ducks, as shown in Table 1. The present study demonstrated that dietary supplementation with *Saccharomyces cerevisiae* influenced feed utilization efficiency rather than growth rate in Pekin ducks during the grower–finisher period (21–42 d). The lower FCR observed in ducks receiving the highest probiotic level (T3) indicates improved feed efficiency, suggesting enhanced nutrient utilization rather than increased growth (Zeng et al. 2022; Liu et al. 2023).

These findings are consistent with previous studies reporting that yeast-based probiotics often improve feed efficiency without significantly altering body weight gain in ducks, particularly during later growth phases when nutrient utilization efficiency becomes more critical than growth rate (Zhang et al. 2021; Gao et al. 2023; Naeem and Bourassa 2025). The improvement in FCR may be attributed to better intestinal function, microbial balance, and metabolic efficiency associated with probiotic supplementation (Abd El-Hack et al. 2020). As shown in Table 2, yeast is rich in amino acids, which are essential components supporting protein synthesis and metabolic processes (Pantaya et al. 2022). The presence of these amino acid components may contribute to improved growth performance and feed efficiency, thereby enhancing overall production performance in ducks.

Dietary inclusion of *Saccharomyces cerevisiae* markedly reduced intestinal populations of pathogenic bacteria, including *Salmonella*, *Shigella*, and *Escherichia coli* (Table 4). Compared with the control group, probiotic-treated ducks exhibited consistently lower pathogen counts, with the greatest reduction observed at higher inclusion levels (T2 and T3). The ability of yeast probiotics to suppress enteric pathogens is well documented and is primarily associated with competitive exclusion, enhancement of gut barrier function, and immune modulation (Markowiak and Śliżewska 2018; Sedghi et al. 2022; Zhang et al. 2021). Mannan-oligosaccharides and β-glucans present in *S. cerevisiae* cell walls can bind pathogenic bacteria, thereby preventing their adhesion to intestinal epithelial cells (Czech et al. 2020; Lee et al. 2021; Abid et al. 2022). This reduction in pathogen load likely contributed indirectly to improved feed efficiency observed in probiotic-treated ducks.

Probiotic supplementation significantly increased high-density lipoprotein (HDL) concentration, while triglycerides, total cholesterol, low-density lipoprotein (LDL), and uric acid levels were not significantly affected (Table 5). The elevation in HDL concentration suggests a favourable modulation of lipid metabolism in probiotic-treated ducks. Similar findings have been reported in poultry, where yeast probiotics improved lipid metabolism by enhancing bile acid deconjugation and regulating hepatic lipid synthesis (Liang et al. 2020; Gao et al. 2025). The absence of significant changes in uric acid concentration indicates that protein metabolism and nitrogen excretion remained stable, which aligns with the lack of significant differences observed in nitrogen retention.

Despite improvements in feed efficiency and gut microbial balance, probiotic supplementation did not significantly affect nitrogen retention, apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), or amino acid digestibility. These results suggest that under the experimental conditions, the basal diet already met the nutritional requirements of Pekin ducks (Kim et al. 2022), limiting the magnitude of probiotic-induced improvements in nutrient digestibility (Poberezhets et al. 2021; Al-Surrayai et al. 2022)

Previous studies have similarly reported inconsistent effects of yeast probiotics on nutrient digestibility, particularly when diets are nutritionally adequate and birds are reared under controlled conditions (He et al. 2021). Therefore, the primary benefits of *S. cerevisiae* in the present study appear to be associated with gut health and feed utilization efficiency rather than direct enhancement of nutrient digestibility. Ammonia concentration in the experimental chamber showed a significant reduction at the

2 h observation point in treatment T2 (Table 7), indicating an early suppression of ammonia production following probiotic supplementation (Jiao et al. 2024). However, this effect was not sustained at later observation times (4–72 hour; Table 7 and 8), where ammonia concentrations did not differ significantly among treatments.

The transient reduction in ammonia may be associated with short-term alterations in nitrogen metabolism and microbial activity following feed incubation, rather than long-term changes in nitrogen excretion patterns (Wang et al. 2025). Importantly, carbon dioxide concentration and chamber temperature remained within acceptable physiological ranges for Pekin ducks throughout the 2–72 hour observation period, indicating that probiotic supplementation did not adversely affect environmental conditions (Ni et al. 2023; Yu et al. 2025). Carbon dioxide emissions showed limited variation among treatments and remained within acceptable thresholds. This observation aligns with previous studies indicating that nutritional interventions more strongly influence ammonia emissions than CO_2 production, which is largely driven by respiration and overall metabolic activity (Zheng et al. 2020; Liu et al. 2023). These findings suggest that while *S. cerevisiae* may contribute to short-term ammonia mitigation, its long-term impact on gaseous emissions under controlled chamber conditions is limited, particularly when nitrogen retention is not significantly altered.

The results indicate that *Saccharomyces cerevisiae* supplementation in Pekin ducks primarily improves feed efficiency and intestinal microbial balance while exerting limited effects on nutrient digestibility and long-term gaseous emissions. The observed reduction in pathogenic bacteria and improvement in HDL concentration highlight the probiotic's role in enhancing gut health and metabolic status, which may contribute to improved production efficiency and animal welfare**.**

Conclusion

In conclusion, the incorporation of the probiotic *Saccharomyces cerevisiae* into the diet of Pekin ducks has demonstrated significant potential in optimising lipid metabolism and reducing harmful gas emissions. The study findings indicate that dietary supplementation with varying levels of this probiotic not only enhances growth performance and improves lipid profiles, but also contributes to a reduction in ammonia emissions, thereby addressing critical concerns related to environmental sustainability in poultry farming. The results suggest that *Saccharomyces cerevisiae* facilitates better nutrient absorption and utilisation, leading to improved overall health and well-being in Pekin ducks. Given the observed benefits, further research is warranted to explain the underlying mechanisms, and to assess the long-term impacts of probiotic supplementation in avian species, ultimately contributing to more sustainable and efficient poultry production practices.

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Author's Contribution: DP: Conceptualisation & Methodology; GS: Data Curation, Writing - Original Draft, Writing - Review & Editing; HS: Visualisation & Supervision; AUP: Project Administration; GA: Funding Acquisition, TFR & MAR: Research Collaborators.

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