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Effects of Dietary Supplementation of *Saccharomyces cerevisiae* on Testosterone, Serum Biochemistry and Performance of ISA Brown Cockerels (*Gallus gallus domesticus*)

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ABSTRACT

This study investigated the effects of dietary *Saccharomyces cerevisiae* (SC) supplementation on testosterone, serum biochemistry, haematology, growth performance, and organ development in ISA Brown cockerels. Fifteen cockerels (six weeks old, 200–300 g) were randomly assigned to three groups designated A, B and C (n=5). Group A served as the control while groups B and C received 5 g/kg and 10 g/kg of SC respectively. Birds were fed standard grower mash for six weeks under uniform conditions. Testosterone was analyzed using a commercial (Enzyme-Linked Immunosorbent Assay (ELISA) kit, while serum biochemistry was assessed with an automated analyzer. Feed and water intake were monitored, and body weight was recorded weekly. Testosterone level was significantly higher ($p<0.05$) in group B. Supplemented groups showed improved urea levels and electrolyte profiles; however, group C had elevated creatinine and AST levels. Platelet counts increased significantly with dosage, while other haematological parameters remained unaffected. Feed intake and weight gain were significantly higher in group C from week 2 to 4 and from week 3 onward, respectively. Testicular weights increased in both supplemented groups, with the highest values in group C. In conclusion, SC supplementation at 5 g/kg improved testosterone level and physiological status, while 10 g/kg enhanced growth and testicular development but may induce metabolic strain. These findings highlight the importance of dose optimization to balance performance benefits with animal health.

Keywords: *Saccharomyces cerevisiae*, ISA brown cockerels, testosterone, serum biochemistry, weight gain, testicular development.

INTRODUCTION

The poultry industry continues to explore nutritional strategies that enhance productivity, health, and reproductive performance in birds. Among these strategies, the use of probiotics has gained significant attention due to their beneficial effects on gut health, immune modulation, and overall physiological status (Gao *et al*., 2008; Das *et al*., 2022). *Saccharomyces cerevisiae*, a non-pathogenic yeast, is one of the most widely studied and utilized probiotics in animal nutrition. Its application in poultry has been associated with improved nutrient digestibility, enhanced intestinal morphology, and a strengthened immune response (Ahiwe *et al*., 2021; Lin *et al*., 2023).

The ISA Brown cockerel, a male counterpart of the popular ISA Brown layer breed, is increasingly recognized in smallholder and commercial systems for its hardiness, adaptability to various environmental conditions, and moderate growth potential (Ekanem *et al*., 2024). While not primarily bred for meat production, ISA Brown cockerels offer economic value through their relatively efficient feed conversion and the potential for dual-purpose use in integrated systems. However, domestication of this breed often reveals reproductive limitations, including suboptimal hormone regulation, delayed testicular development, and inconsistent fertility rates—likely due to genetic selection pressures focused on egg production traits in females. These challenges reduce the reproductive efficiency of the males and limit their utility in sustainable breeding programs. Additionally, ISA Brown cockerels may exhibit slower growth rates and vulnerability to stress-related metabolic imbalances when compared to broiler strains. These limitations underscore the need for nutritional strategies that enhance both growth and reproductive performance. Probiotic formulations such as *S. cerevisiae* offer a promising avenue, as they have been shown to modulate gut microbiota, improve nutrient absorption, and influence endocrine responses.

Specifically, *S. cerevisiae* may help optimize the hypothalamic–pituitary–gonadal axis, improve testicular function, and enhance overall physiological resilience. Given the paucity of targeted research on ISA Brown cockerels, evaluating the impact of *S. cerevisiae* supplementation on their hormonal, metabolic, and growth profiles is essential to unlocking their full productive potential and ensuring more efficient utilization within poultry systems.

In male poultry, reproductive success is largely governed by the integrity of the hypothalamic–pituitary–gonadal (HPG) axis and the activity of testicular steroidogenesis (Yan *et al*., 2025). Hormones such as LH, FSH and testosterone are crucial for the regulation of spermatogenesis and secondary sexual characteristics. Several studies have suggested that dietary interventions, including probiotics, may influence these endocrine pathways by altering the host's metabolic status and modulating stress responses (Olugbemi *et al*., 2010). However, there is limited empirical evidence on the specific effects of *S. cerevisiae* supplementation on the reproductive hormonal milieu and organ development in cockerels.

Beyond reproduction, probiotic supplementation may also impact biochemical and haematological parameters, which serve as indicators of organ function and systemic health. Parameters such as serum creatinine, urea, liver enzymes, and lipid profiles offer insights into the metabolic and hepatic responses to dietary treatments (Shehzadi *et al*., 2023; Amoah *et al*., 2023). Additionally, feed intake, water consumption, and body weight gain are critical performance indicators influenced by dietary yeast, possibly due to improved palatability and gastrointestinal function (Lawrence-Azua *et al*., 2018; Zhu *et al*., 2023).

Specifically, the study aimed to determine the effects of *S. cerevisiae* on testosterone, blood chemistry, haematological indices, growth performance, feed and water intake, and relative organ weights of cockerels.

MATERALS AND METHODS

Experimental Design and Animal Management
The study was conducted using fifteen (15) healthy ISA Brown cockerels aged 6 weeks old and weighing 200-300g. They were randomly assigned into three dietary treatment groups designated A, B and C (n=5). The birds were fed standard pelleted grower mash diets for six weeks under standard management conditions. Group A served as the control and received a basal diet without the supplement, group B received basal diet supplemented with *S. cerevisiae* at 5 g/kg of feed, and group C received 10 g/kg of *S. cerevisiae* in their diet. Birds were housed individually in well-ventilated cages under standard management conditions and had *ad libitum* access to feed and water throughout the 6-week experiment period.

Determination of Feed Intake

Feed intake was monitored weekly by providing a known quantity of feed daily and measuring the leftover at the end of each 24-hour period. Weekly feed intake was calculated by subtracting the remaining feed from the total feed offered. Feed spillage was minimized by using specially designed feeding troughs and was not included in intake calculations. Weekly averages were computed and expressed in grams per bird.

Determination of Water Intake

Water consumption was recorded weekly using graduated water containers calibrated in milliliters. Each bird was provided with 1000 mL of water daily and the volume of leftover water was recorded after 24 hours. Weekly water consumption was calculated as the average of daily intakes over 7 days and recorded in milliliters.

Body Weight Measurement

Weekly weight of the individual bird in each group was measured using a digital weighing scale with 0.01kg sensitivity. Weight gain was determined by subtracting the initial weight from the final weight for that week. Average of the weights of the birds in each group was then calculated and recorded in grams.

Determination of Haematological Profile

Whole blood samples were collected into ethylenediaminetetraacetic acid (EDTA)-coated tubes and analyzed for haematological parameters such as red blood cell (RBC) count, haemoglobin (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), total leucocyte count (TLC), neutrophils (NEU), lymphocytes (LYM), and monocytes (MON). Analyses were performed using an automated haematology analyzer (Orphee mythic 18, Japan).

Determination of Testosterone Concentration

5 mL of blood sample was collected via the wing vein of each bird using sterile syringes. A total of 15 samples were collected at about 11:00 am. Samples were allowed to clot and then centrifuged at 3000 revolution per minute (rpm) for 10 minutes to harvest serum. The serum was divided into three portions and preserved at -80^{o}C until analyzed.

Serum testosterone was determined using commercial Enzyme Linked Immunosorbent Assay (ELISA) (Monobind Inc., Lake Forest, USA). Briefly, 50 µL of standard, sample serum, or control were dispensed into the well, followed by an addition of 100 µL of enzyme conjugate. After incubation for 1 hour at room temperature, wells were rinsed with diluted washing solution three times. Next, 150 µL of the substrate solution were added into the well and incubated for 15 minutes at room temperature. Measurement of testosterone concentration was performed at 450 nm in ELISA reader (Microplate Autoreader, BioRad Laboratory, Hercules, CA, USA) after adding the stop solution. All testing materials were triplicated for the assay. The sensitivity and intra- and inter-assay variation coefficients of the kit were 0 to 0.083 ng/mL, 3.28% to 4.16%, and 4.73% to 9.94%, respectively.

Determination of Serum Biochemical Profile

Serum aliquots from all the animals in each of the groups (15 samples all together) were used to evaluate biochemical parameters such as electrolytes (sodium, potassium, chloride, and bicarbonate), urea, creatinine, liver enzymes [alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase

(AST)], bilirubin levels (total and direct), total cholesterol (Tchol), triglycerides (Trig), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TPROT), and albumin (ALB). These analyses were carried out using an automated chemistry analyzer (Berckman Coulter Synchron CX9 PRO Automated Biochemistry analyzer), calibrated according to the supplier's specifications

Relative Organ Weights (Organo-somatic Index) Determination

At the end of the trial, the birds in each group were humanely euthanized through cervical dislocation and dissected to collect internal organs including liver, heart, spleen, lungs, pancreas, and testes. Each organ was carefully excised, blotted dry, and weighed using a digital precision scale. Relative organ weight was calculated as percentage of final body weight according to the formula (Sellers *et al*., 2007).

$$OSI = \frac{Organ\ weight}{Weight\ of\ Animal} \times 100$$

Statistical Analysis

The data obtained in this study were subjected to One-way Analysis of Variance (ANOVA) using SPSS version 22.0. Turkey post hoc test was used to separate the mean between the groups. Differences between the means were considered statistically significant at $p<0.05$. Results were presented as Means ± Standard Error of Mean (SEM). Superscripts (a, b, c) were used to indicate significant differences between treatment groups.

Ethical Statement

This study was conducted in accordance with internationally accepted principles for the ethical use and care of animals in research, as outlined by the World Organization for Animal Health (OIE) Terrestrial Animal Health Code and the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011). All experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of university of Abuja, under the protocol number IACUC/REF/2025/0421.

RESULTS

Feed Intake (g) of ISA Brown Cockerels Fed with Graded Doses of *Saccharomycese cerevisiae* in Diet

Table 1 shows the result of the effect of *S. cerevisiae* supplementation on feed intake of cockerels. Cockerels in group C (10 g/kg SC) consumed significantly more feed than groups A and B during weeks 2 to 4 ($p < 0.05$). In Week 2, group C (986.9 ± 9.31 g) significantly outperformed groups A (827.4 ± 29.07 g) and B (861.4 ± 37.28 g). Similar trends were observed in Weeks 3 and 4. However, feed intake differences were not statistically significant in Weeks 1, 5 and 6.

Table 1: Feed Intake (g) of ISA Brown Cockerels Fed with Graded Doses of *Saccharomycese cerevisiae* in Diet

Feeding Duration (Weeks)	Group A (control)	Group B (5g/kgSC)	Group C (10g/kgSC)
WEEK 1	733.1±35.98	746.1±34.33	857.3±37.87
WEEK 2	827.4±29.07[a]	861.4±37.28[a]	986.9±9.31[b]
WEEK 3	702.1±42.25[a]	885.0±14.25[a]	969.1±11.00[b]
WEEK 4	631.9±74.05[a]	712.0±33.61[a]	942.6±24.76[b]
WEEK 5	697.4±51.51	655.0±52.23	634±7 5.61
WEEK 6	761.3±77.87	638.0±39.94	695.8±23.11

Means value with different superscripts [a,b] in a row indicate significant differences at $p < 0.05$.

Water Intake (mL) of ISA Brown Cockerels Fed with Graded Doses of *Saccharomycese cerevisiae* in Diet

The effect of *S. cerevisiae* supplementation on water intake of cockerels are shown in table 2. There were no statistically significant differences ($p > 0.05$) in water intake among the groups throughout the 6-week period. Although group B consistently showed slightly higher weekly water consumption from Weeks 3 to 5, these changes were not significant when compared to groups A and C, indicating that SC supplementation did not significantly alter water intake patterns in cockerels.

Table 2: Water Intake (mL) of ISA Brown Cockerels Fed with Graded Doses of *Saccharomycese cerevisiae* in Diet

Water Intake Duration (Weeks)	Group A (Control)	Group B (5g/kgSC)	Group C (10g/kgSC)
WEEK 1	4573±374.20	4657±432.32	4754±324.23
WEEK 2	4634±376.76	4743±543.21	4654±426.33
WEEK 3	4629±338.61	5786±545.32	5271±413.91
WEEK 4	4900±466.04	5229±467.91	4786±448.02
WEEK 5	4571±335.03	5271±337.93	4957±351.14
WEEK 6	6133±539.55	5933±495.12	5250±463.16

Table 3: Weight (g) of ISA Brown Cockerels Fed with Graded Doses of *Saccharomyces cerevisiae* Supplementation in Diet

Weight (g)	Group A (control)	Group B (5g/kgSC)	Group C (10g/kgSC)
WEEK 1	1.32±0.06	1.54±0.10	1.73±0.17
WEEK 2	1.51±0.13	1.77±012	2.07±0.17
WEEK 3	1.70±0.06[a]	1.95±0.07[a]	2.28±0.04[b]
WEEK 4	1.77±0.01[a]	2.07±0.05[a]	2.41±0.09[b]
WEEK 5	1.82±0.04[a]	2.14±0.02[a]	2.52±0.02[b]
WEEK 6	1.86±0.01[a]	2.13±0.03[a]	2.58±0.04[b]

Means value with different superscripts [a,b] in a row indicate significant differences at $p < 0.05$.

Weight (g) of ISA Brown Cockerels Fed with Graded Doses of *Saccharomyces cerevisiae* Supplementation in Diet

Table 3 shows the effect of *S. cerevisiae* supplementation on weight gain of cockerels. Significant differences ($p < 0.05$) in body weight gain were observed from Week 3 onward. Group C consistently recorded higher weight gains than groups A and B. For instance, in Week 6, Group C (2.58 ± 0.04 kg) significantly surpassed group A (1.86 ± 0.01 kg) and group B (2.13 ± 0.03 kg). Similar significant differences were evident in Weeks 3 to 5. These results confirm the growth-promoting effect of higher *S. cerevisiae* inclusion.

Haematological Profile of ISA Brown Cockerels Fed with Graded Doses of *Saccharomyces cerevisiae* in Diet

Table 4 shows the effects of *S. cerevisiae* supplementation on haematological parameters of cockerels. Among all measured haematological parameters, only platelet (PLT) count showed significant differences ($p < 0.05$). Group C exhibited the highest platelet count (174.50 ± 55.39 $\times 10^6/\mu L$), followed by group B (115.30 ± 11.24 $\times 10^6/\mu L$), while the control had the lowest value (72.20 ± 5.73 $\times 10^6/\mu L$). Other parameters such as red blood cell count (RBC), haemoglobin (HB), packed cell volume (PCV), and white blood cell subsets showed no statistically significant ($p > 0.05$) variation across the treatment groups, indicating overall haematological stability with yeast supplementation.

Table 4: Haematological profile of ISA Brown Cockerels Fed with Graded Doses *Saccharomycese cerevisiae* in Diet

Parameters	Group A (control)	Group B (5g/kgSC)	Group C (10g/kgSC)
RBC ($x10^3/\mu L$)	2.50±0.04	2.41±0.16	2.61±0.20
Hb (g/dL)	12.00±0.20	11.85±0.846	12.10±1.17
PCV (%)	35.80±0.48	36.60±2.40	37.26±3.02
MCV (fl)	142.80±0.72	148.90±1.91	142.30±2.85
MCH (Pg)	47.90±0.34	48.72±0.53	46.05±1.28
MCHC (g/dL)	33.52±0.162	32.68±0.23	32.32±0.41
PLT ($x10^6/\mu L$)	72.20±5.73[a]	115.30±11.24[b]	174.5±55.39[c]
NEU ($x10^3/\mu L$)	3.800±0.58	6.720±1.90	5.0±1.00
LYM ($x10^3/\mu L$)	67±15.78	78.07±4.44	80.±2.84
MON ($x10^3/\mu L$)	13.60±0.678	14.6±2.61	15.00±1.92
TLC ($x10^6/\mu L$)	117.6±0.68	111.4±2.30	115.64±2.16

Means value with different superscripts [a,b] in a row indicate significant differences at $p < 0.05$.

Testosterone Concentration and Serum Biochemical Profile of ISA Brown Cockerels Fed with Graded Doses of *Saccharomyces cerevisiae* in Diets.

Results of the effect of graded doses of *S. cerevisiae* supplementation on testosterone concentration and serum biochemical profile of cockerels are presented in Table 5. The supplement significantly influenced the serum concentrations of testosterone in cockerels. Testosterone concentration was significantly higher ($p < 0.05$) in group B (3.97 ± 3.75 ng/mL) than in group A (0.14 ± 0.05ng/mL) and group C (0.39 ± 0.23 ng/mL).

Significant differences ($p < 0.05$) were also observed in several biochemical parameters among the treatment groups and the control group. Urea levels were significantly lower in groups B (1.05 ± 0.04 mmol/L) and C (0.98 ± 0.06 mmol/L) compared to the control (1.25 ± 0.07 mmol/L), while creatinine was significantly elevated in group C (6.02 ± 3.10 mmol/L) compared to groups A (1.70 ± 0.77 mmol/L) and B (2.10 ± 0.39 mmol/L). Aspartate aminotransferase (AST) activity was also significantly higher in group B (318.60 ± 20.82 IU/L) than in groups A (165.20 ± 21.06 IU/L) and C (209.60 ± 16.38 IU/L). No significant differences were observed in ALT, ALP, cholesterol, triglycerides, or protein parameters, although minor trends were noted across the groups.

Table 5: Testosterone Concentration and Serum Biochemical Profile of ISA Brown Cockerels Fed with Graded Doses of *Saccharomyces cerevisiae* in Diets

Parameters	Group A (Control)	Group B (5g/kgSC)	Group C (10g/kgSC)
Testosterone (ng/ml)	0.14±0.054[a]	3.97±3.75[b]	0.39±0.23[a]
Na (mmol/L)	150.0±2.02	160.60±1.50	153.50±3.61
K(mmol/L)	3.780±0.058	5.70±0.54	3.36±0.357
Cl(mmol/L)	114.2±1.96	116.6±1.43	117.6±1.32
Urea(mmol/L)	1.250±0.07[a]	1.048±0.04[b]	0.978±0.06[b]
Cr(mmol/L)	1.700±0.77[a]	2.100±0.39[a]	6.020±3.10[b]
Tchol(mmol/L)	2.85±0.177	2.52±0.067	2.77±0.22
Trig(mmol/L)	0.214±0.030	0.28±0.037	0.50±0.15
HDL(mmol/L)	1.814±0.10	1.352±0.08	1.44±0.04
LDL(mmol/L)	0.96±0.067	1.02±0.01	1.122±0.20
TBL(mmol/L)	1.66±0.20	2.03±0.04	1.86±0.27
DBL(mmol/L)	1.00±0.15	1.34±0.36	1.12±0.037
ALP(IU/L)	1518±193.8	1090±38.43	1030±170.1
AST(IU/L)	165.2±21.06[a]	318.6±20.82[b]	209.6±16.38[a]
ALT(IU/L)	16.80±1.28	18.00±1.92	15.30±0.830
TPROT(g/L)	30.40±2.54	32.00±1.789	35.60±1.86
ALB(g/L)	14.80±0.80	15.32±0.80	18.00±1.00

Means value with different superscripts [a,b] in a row indicate significant differences at $p < 0.05$.

Na= Sodium, K= Potassium, Cl= Chlorine, HCO= Bicarbonate, UREA= Urea, Cr= Creatinine, TBL= Total Bilirubin, DBL= Direct bilirubin, ALP= Alkaline Phosphate, AST=Aspartate Aminotransferase, ALT= Alanine Aminotransferase, TPROT= Total protein, ALB= Albumin, TCHOLE= Total Cholesterol, TRIG= Triglycerol, HDL= High Density Lipo-Protein, LDL= Low Density Lipoprotein

Relative Organ Weights (Organo-Somatic Indices) of ISA Brown Cockerels Fed with *Saccharomyces cerevisiae* in Diet

Table 6 shows the effect of *S. cerevisiae* on relative organ weights (organo-somatic indices) of cockerel following supplementation with *S. cerevisiae.* Among the examined organs, only the testes showed significant differences ($p < 0.05$) among treatment groups. Testicular weights increased progressively with higher *S. cerevisiae* supplementation: Group A (2.15 ± 0.19 g), group B (3.34 ± 0.46 g), and Group C (5.03 ± 0.46 g), indicating a dose-responsive effect on testicular development. Other organ weights, including liver, spleen, lungs, pancreas, and heart, did not differ significantly ($p > 0.05$) although numerical variations were observed.

Table 6: Relative Organ Weights (Organo-Somatic Indices) of ISA Brown Cockerels Fed with *Saccharomyces cerevisiae* in Diet

Organs	Group A (control)	Group B (5g/kgSC)	Group C (10g/kgSC)
Spleen(g)	0.11±0.01	0.11±0.00	0.11±0.01
Lung (g)	0.52±0.02	0.51±0.05	0.42±0.05
Liver (g)	1.52±0.09	2.12±0.37	1.90±0.24
Pancreas (g)	0.154±0.01	0.140±0.01	0.170±0.01
Testes (g)	2.15±0.19[a]	3.34±0.46[b]	5.03±0.46[c]
Heart (g)	0.53±0.03	0.41±0.00	0.53±0.04

Means value with different superscripts [a,b,c] in a row indicate significant differences at $p < 0.05$.

DISCUSSION

The observation that cockerels in the 10 g/kg *Saccharomyces cerevisiae* group consumed more feed during the early weeks, possibly due to increased appetite or improved palatability, aligns with studies reporting that yeast supplementation can enhance feed intake, particularly during the initial stages of supplementation. Yeast products are known to improve the palatability of feed by increasing the availability of nutrients, which may stimulate appetite and encourage greater feed consumption (Liu *et al*., 2016). However, the diminishing effect on feed intake in later weeks suggests an adaptation or physiological limit, which has been observed in studies where animals adjust to nutrient-rich diets or reach a point of satiation (Hernández *et al*., 2009). This phenomenon of initial increased consumption followed by a plateau or decline is common in supplementation trials, reflecting the body's capacity to adjust to nutrient intake over time.

Enhanced feed intake during earlier growth phases may have contributed to the increased weight gain observed in groups B and C. Probiotic yeast is known to improve gut morphology and enzymatic activity, thereby stimulating appetite and nutrient use efficiency (Gao *et al*., 2008; Agbonu and Aka, 2016; Amenyogbe *et al*., 2024).

Despite the difference in feed intake, water consumption did not significantly differ across groups from weeks 3 to 6. This indicates that water intake was not directly influenced by *S. cerevisiae* supplementation, suggesting the observed differences in weight gain and metabolism were more likely driven by feed composition and nutrient

assimilation rather than hydration status. However, a trend toward increased water intake in *S. cerevisiae*-fed groups was noted, particularly in weeks 3 to 5, which may correspond to higher metabolic activity during this period.

Weight gain patterns across the 6-week period clearly demonstrate the growth performance-enhancing effect of *S. cerevisiae* with group C showing significantly higher weekly weight gains from week 3 onward. This may be attributed to improved protein digestibility and gut health facilitated by yeast supplementation (Mohammadi *et al*., 2020). In another study, Agbonu and Aka (2016); Agbonu *et al*. (2016) reported enhanced weight gain in West African dwarf sheep due to increased nutrients digestibility, feed conversion ratio, feed efficiency and reduced enteric methane production following feed supplementation with *S. cerevisiae*. Similarly, Hossain *et al*. (2025) also reported enhanced growth performance in broilers following supplementation of their feed with *S. cerevisiae* by modulating gut microbiota and immune function. Importantly, group B (5 g/kg SC) also performed better than the control group, hence highlight the benefits of moderate supplementation *S. cerevisiae* for poultry productivity without overstimulation.

The results of this study demonstrated a clear impact of *S. cerevisiae* supplementation on testosterone concentration of ISA brown cockerels. Cockerels fed 5 g/kg SC (group B) had significantly elevated level of testosterone compared to both the control (group A) and the higher-dose group (group C). The observed increase in testosterone levels at a dose of 5 g/kg bw in ISA Brown cockerels can be attributed to the potential biphasic or hermetic effect of the supplement, where lower doses stimulate physiological responses like testosterone production, while higher doses may overwhelm the body's endocrine regulation mechanisms (Calabrese and Baldwin, 2001). At 5 g/kg, the supplement might enhance steroidogenesis by modulating enzymes involved in testosterone biosynthesis, potentially via the hypothalamic-pituitary-gonadal (HPG) axis (Veldhuis *et al*., 1995). However, at 10 g/kg, the dose could induce a negative feedback loop or alter metabolic pathways, leading to reduced testosterone secretion due to overstimulation or toxicity, as high doses may suppress gonadotropin-releasing hormone (GnRH) release or interfere with receptor sensitivity (Kumar *et al*., 2019). This dose-dependent pattern is commonly observed in endocrinological studies, where substances exhibit different effects at varying concentrations (Sharma *et al*., 2017).

Biochemical analysis further supports the physiological relevance of *S. cerevisiae* supplementation. Serum urea levels were significantly reduced in both *S. cerevisiae* fed groups, indicating improved protein metabolism or renal clearance. This aligns with the findings of Mohammadi *et al*. (2020), who reported yeast supplementation in poultry, has been linked to improved renal clearance by enhancing the detoxification processes in the kidneys; likely facilitated by the modulation of nitrogen excretion pathways, which contributes to lower serum urea concentrations.

The finding that the 5 g/kg *Saccharomyces cerevisiae* supplementation improved electrolyte balance, with elevated sodium, potassium, and chloride levels, supports previous studies suggesting that yeast supplementation enhances mineral absorption by improving gut health and microbial activity. This finding aligns with the established role of *S. cerevisiae* in enhancing nutrient absorption through its prebiotic effects (Kluge *et al*., 2006; Yin *et al*., 2010). In relation to liver enzymes, stable or normal levels of AST, ALT, and ALP would indicate that the liver is functioning properly without undue stress, which is consistent with the beneficial effects of yeast supplementation on liver and kidney function (Zhang *et al*., 2014). Overall, these results suggest that *S. cerevisiae* at 5 g/kg supports both electrolyte homeostasis and efficient mineral metabolism without overloading liver function. These changes highlight the importance of dose optimization in dietary supplementation.

The haematological indices demonstrated that platelet count (PLT) increased significantly in groups B and C peaking in the 10 g/kg SC group. Although the red and white blood cell parameters were within normal ranges, the platelet surge could suggest enhanced haematopoiesis or immunomodulatory effects of *S. cerevisiae*. This aligns with previous which indicate that yeast-derived β-glucans stimulate bone marrow activity and leukocyte function (Thomas *et al.,* 2022). Notably, neutrophil and lymphocyte counts increased moderately in the supplemented groups, suggesting improved immune surveillance, though the differences were not statistically significant.

Finally, the relative organ weights offer valuable insights into the physiological effects of *S. cerevisiae*. While the weights of the spleen, lung, liver, pancreas, and heart showed no significant changes, a notable increase in testicular weight was observed in groups B and C. This increase aligns with the elevated testosterone levels, suggesting that *S. cerevisiae* may promote enhanced testicular development and spermatogenic activity. This finding is consistent with previous studies, which have indicated a positive influence of *S. cerevisiae* on reproductive health and hormone regulation (Smith *et al*., 2020). Increased testicular sizes linked to improved fertility in poultry and may result from direct hormonal stimulation or improved nutritional status (Fouad *et al*., 2020). These findings underscore the reproductive benefits of yeast supplementation, particularly at higher doses, though careful monitoring of metabolic indicators is warranted.

Conclusion

In conclusion, this study demonstrated that adding *Saccharomyces cerevisiae* to the diets of cockerels, especially at the moderate level of 5 g/kg, was beneficial.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contribution

AOA was involved in conceptualization, formal analysis, investigation, methodology, supervision, and writing the original draft and editing. IJ was involved in investigation, methodology, writing, reviewing and editing.

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