

Effects of dietary supplementation with *Saccharomyces cerevisiae* on the growth performance and pathology of broiler chickens infected with mixed *Eimeria* species

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Abstract

This study investigated the effects of *Saccharomyces cerevisiae* (SC) supplementation on growth performance and pathological changes associated with mixed *Eimeria* infection in broiler chickens. Seventy-two day-old chicks were randomly assigned to two primary groups: Group 1 (unsupplemented) and Group 2 (supplemented with 1% SC in feed). At three weeks post-hatch, each primary group was subdivided into two: Group 1 was subdivided into Unsupplemented–unchallenged (Group A) and Unsupplemented–challenged (Group C); and while Group 2 was subdivided into Supplemented–challenged (Group B) and Supplemented–unchallenged (Group D). Groups B and C were orally challenged with *Eimeria* oocysts at week 3 of age. Results showed that feed intake was significantly higher ($P < 0.05$) in unchallenged groups (A and D) than in challenged groups (B and C). Although group D had higher feed intake than group A, the difference was not statistically significant. Body weight followed a similar trend, with unchallenged groups (A and D) outperforming challenged groups (B and C); group D was significantly heavier ($P < 0.05$) than group A on days 5, 10, and 30 post-challenge (PC) with *Eimeria* oocysts. By day 35, group D exhibited the greatest body weight gain among all groups. Morbidity was recorded in all groups, peaking on day 15 PC in challenged groups, while group D showed minimal morbidity on day 20 PC. Gross intestinal lesions were more pronounced in challenged birds (B and C) compared to unchallenged birds (A and D). No mortality occurred. Histopathological evaluation revealed moderate intestinal damage, including villous necrosis and loss, in challenged groups, whereas unchallenged birds retained normal mucosal architecture. Mild to moderate mucosal inflammation and congestion were observed in all groups over time. The findings indicate that SC supplementation (1 g/kg feed) enhanced growth performance in unchallenged broilers and mitigated morbidity and severity of intestinal lesions in supplemented challenged birds, though it did not provide complete protection. Dietary inclusion of SC is recommended to improve productivity and as an aid in the management of *Eimeria* infections in broilers.

Keywords: Coccidiosis; *Eimeria* spp.; *Saccharomyces cerevisiae*; Dietary supplementation; Broiler chickens.

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Introduction

Coccidiosis is a major disease of economic importance in poultry business. The disease is caused by organisms of the genus *Eimeria*, a protozoan, which multiplies in the mucosal epithelium of the chicken's intestine, causing tissue damage, with resultant interruption of the feeding and digestive processes that may lead to morbidity, loss of production and mortality (Gerhold, 2023). Although there are other methods of diagnosis of *Eimeria* infection in chickens, loss of weight and intestinal lesions are important determinants of the infection (Fatoba and Adeleke, 2018).

Control and treatment of *Eimeria* infection are by vaccination and use of anti-coccidial agents (Gerhold, 2023; Ahmad et al., 2024), however, current reports of vaccination failures and resistance of *Eimeria* species to anti-coccidial agents (Flores et al., 2022) have necessitated a demand for additional methods of control.

Saccharomyces cerevisiae (SC), a probiotic, widely commercialized and used in poultry as an effective adsorbent and growth promoter (Pang et al., 2022), has earlier been evaluated for the treatment of *Eimeria* infection in chickens (Soutter et al., 2022); however, the findings were inconclusive, providing no definitive evidence of either efficacy or ineffectiveness. The present study evaluated the effects of SC supplementation on the growth performance and pathology caused by infection with *Eimeria* spp in broiler chickens.

Materials and Methods

Flock History/Management: Seventy-two day-old broiler chicks (Ross® 2000), procured from a certified hatchery in Ibadan, Oyo state Nigeria, were used for the study. The birds were vaccinated against Infectious bursal and Newcastle diseases according to local recommendation (Ekiri et al., 2021). The chicks were housed on deep litter at the Veterinary

Teaching and Research Farm, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Brooding temperature was maintained at 29 – 32°C for the first one week and regulated on the basis of the ambient temperature. The birds were fed *ad-libitum* using appropriate broiler rations all through the experiment (Deaton et al., 1996). The use and care of the birds was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (Approval Reference Number: FVM-UNN-IACUC-2021-0467).

Grouping of the experimental birds: The 72 broiler chicks were initially randomly assigned to two groups (Group 1 and 2) of 36 birds each. At three weeks post-hatch, birds in Group 1 were randomly subdivided into Groups A and C of 18 birds each, while Group 2 birds were also subdivided into Groups B and D of 18 birds each.

Feeding of experimental birds with the probiotic supplement (SC): The experimental diet was compounded based on the basic rules and nutritional standards, and analyzed using the AOAC method, with addition of the probiotic, *Saccharomyces cerevisiae* MG865964 strain at 1g/kg in the diet compounded for broiler chickens in Groups B and D and fed to them all through the experimental period (Table 1). Birds in Groups A were the Unsupplemented-unchallenged control, while those in Group B received feed supplemented with *Saccharomyces cerevisiae* and were challenged with *Eimeria* infection. Group C birds were the Unsupplemented challenged group, while Group D birds were the Supplemented unchallenged. Strict biosecurity was observed including changing of litter materials on daily basis. Fecal samples were collected from all experimental birds daily in the 24 hours preceding *Eimeria* challenge, to monitor baseline oocyst shedding. This approach followed established protocols (Su et al., 2025).

Preparation of the *Eimeria* species inoculum:

Eimeria oocysts were obtained from naturally infected chickens by the method described by (Gadelhaq et al., 2015). Five chickens infected with *Eimeria* species were obtained from a reported case of coccidiosis. The birds were placed in a large plastic bath, and a lid made of wire mesh was placed on the top of the bath to allow for ventilation. Faecal samples excreted by the birds were collected and placed in a polythene bag packed with ice and taken to the parasitology laboratory for processing.

Experimental challenge: Doses of 1×10^4 sporulated oocysts (Figure 1) in 1.5 ml of distilled water (Brito et al., 2014) were administered per os to each of all the challenged groups (Groups B and C), while

each of the unchallenged groups (Groups A and D) received 1.5 ml of sterile water.

Data collection: Body weights of birds in all the groups were determined on days 0, 5, 10, 15, 20, 25, 30, and 35 post challenge (PC). After the first appearance of oocysts from fresh faecal sample, determination of the rate of oocysts shedding per gramme of faeces was carried out at 5 days interval, using the floatation method. Birds in all the groups were observed twice daily for clinical signs and mortality from days 0 – 35 PC, while necropsy findings and histopathological changes were recorded on birds for all the groups from different segments of the intestine on dead or sacrificed birds on days 10, 25 and 35 PC following standard procedure (Amer et al., 2010).

Table 1. Proximate composition of the two sets of feeds given to the broilers chickens used for the study: the unsupplemented feed was given to Groups A and C, and the supplemented feed was given to Groups B and D.

Nutrient	Percentage/proportion of nutrient in the feed	
	Unsupplemented feed	Supplemented feed
Crude protein	17.75 %	17.76 %
Fat	8.50 %	8.60 %
Moisture	7.25 %	7.35 %
Fibre	4.85 %	4.95 %
Ash	9.92 %	10.53 %
Energy (cal/kg)	3162.6 cal/kg	3153.16 cal/kg

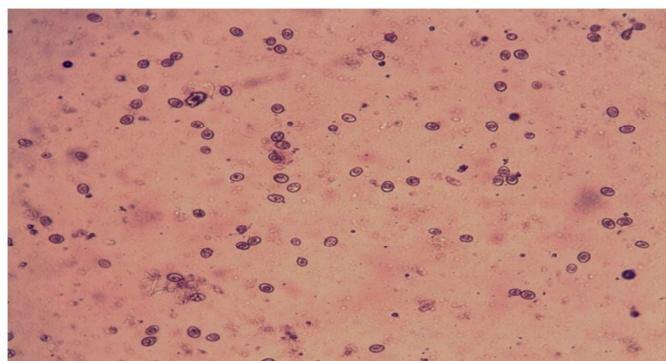


Figure 1. Mixed sporulated *Eimeria* oocysts used for the experimental challenge infection [x100]

Data Analysis: Growth performance parameters (feed intake, body weight, body weight gain, and feed conversion ratio) were analyzed using a two-way analysis of variance (ANOVA) with dietary treatment (*Saccharomyces cerevisiae* supplementation versus no supplementation) and challenge status (*Eimeria* challenged versus unchallenged) as fixed factors. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) prior to analysis. Where a significant interaction or main effect was detected, means were separated using Tukey's Honest Significant Difference (HSD) post-hoc test. Ordinal data, including intestinal gross lesion scores, were analyzed using the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni adjustment. Morbidity data

(counts) were compared using the Chi-square test where appropriate. All analyses were performed using SPSS version XX, GraphPad Prism version XX and significance was accepted at $p < 0.05$. Data were presented as mean \pm standard error of the mean (SEM) unless otherwise stated.

Results

From days 5 to 30 post-challenge (PC), feed intake was significantly higher ($p < 0.05$) in the unsupplemented-unchallenged (A) and supplemented-unchallenged (D) groups compared with the supplemented-challenged (B) and unsupplemented-challenged (C) groups (Table 2), suggesting that *Eimeria* challenge reduced feed consumption irrespective of dietary supplementation.

Table 2. Feed intake (g) of broiler chickens fed diets supplemented with *Saccharomyces cerevisiae* (SC) and challenged with mixed *Eimeria* oocysts infection.

Days post infection (days)	Group A (Unsupplemented unchallenged)	Group B (Supplemented challenged)	Group C (Unsupplemented challenged)	Group D (Supplemented unchallenged)
Day 0	491.72 ^{a c} (56.12)	393.06 ^{a b} (11.64)	385.06 ^{a b} (29.19)	552.78 ^c (22.51)
Day 5	1154.89 ^a (57.77)	949.17 ^b (7.98)	948.62 ^b (40.30)	1241.89 ^a (19.77)
Day 10	1920.61 ^a (81.23)	1546.08 ^b (32.81)	1517.13 ^b (30.80)	1936.05 ^a (29.60)
Day 15	2744.39 ^a (124.88)	2232.06 ^b (52.01)	2173.96 ^b (81.01)	2760.49 ^a (28.36)
Day 20	3632.16 ^a (185.67)	3038.95 ^b (68.06)	2930.63 ^b (120.65)	3618.10 ^a (74.56)
Day 25	4576.27 ^a (241.60)	3856.56 ^b (132.03)	3801.68 ^b (196.79)	4533.27 ^a (95.64)
Day 30	5635.77 ^a (287.82)	4689.66 ^b (152.06)	4696.83 ^b (253.33)	5367.10 ^{a b} (154.18)
Day 35	491.72 ^{a c} (56.12)	393.06 ^{a b} (11.64)	385.06 ^{a b} (29.19)	552.78 ^c (22.51)

Values are presented as means, with SEM in brackets; Mean values bearing different superscripts in the same row differ significantly ($p < 0.05$).

Table 3. Body weights (g) of broiler chickens fed diets supplemented with *Saccharomyces cerevisiae* (SC) and challenged with mixed *Eimeria* oocysts infection.

Days post-infection (days)	Group A (Unsupplemented unchallenged)	Group B (Supplemented challenged)	Group C (Unsupplemented challenged)	Group D (Supplemented unchallenged)
Day 0	700.11 ^a (33.63)	610.78 ^b (5.18)	585.06 ^b (35.94)	768.11 ^a (21.47)
Day 5	916.81 ^a (41.38)	743.72 ^b (16.97)	712.39 ^b (43.91)	1033.11 ^c (30.76)
Day 10	1255.55 ^a (49.49)	936.17 ^b (14.06)	908.50 ^b (55.43)	1421.22 ^c (22.18)
Day 15	1600.94 ^a (77.69)	1149.61 ^b (33.39)	1113.17 ^b (36.05)	1696.72 ^a (40.30)
Day 20	1967.78 ^a (179.99)	1534.94 ^b (79.92)	1333.00 ^b (96.75)	2059.50 ^a (85.29)
Day 25	2385.33 ^a (207.26)	1784.17 ^b (162.61)	1672.67 ^b (125.14)	2483.89 ^a (110.21)
Day 30	2642.11 ^{a,c} (205.69)	2087.11 ^{a,b} (208.79)	1956.22 ^b (130.57)	2726.78 ^c (170.23)
Day 35	2726.54 ^a (84.24)	2308.50 ^b (115.80)	2120.61 ^b (173.15)	2984.45 ^a (96.03)

Values are presented as means, with SEM in brackets; Mean values bearing different superscripts in the same row differ significantly ($p < 0.05$).

From baseline to days 25 and 35 PC, body weight was significantly higher ($p < 0.05$) in groups A and D than in groups B and C (Table 3), reflecting the combined influence of higher feed intake and absence of parasitic stress. On days 5, 10, and 30 PC, group D recorded significantly higher body weight ($p < 0.05$) than all other groups, indicating a possible synergistic effect of *Saccharomyces cerevisiae* supplementation and absence of *Eimeria* infection on growth.

Body weight gain from baseline to day 20 PC was also significantly greater ($p < 0.05$) in groups A and D compared with groups B and C (Table 4). On day 35 PC, group D achieved the greatest body weight gain ($p < 0.05$) compared with all other groups (Table 4), further supporting the beneficial role of

supplementation under non-challenged conditions.

Feed conversion ratio (FCR) from baseline to days 10 and 35 PC was significantly lower ($p < 0.05$) in groups A and D compared with groups B and C (Table 5), indicating superior feed efficiency in the unchallenged birds. The higher FCR in challenged groups was consistent with reduced feed intake and body weight gain observed in these birds.

No morbidity was observed in any group from day 0 to 9 PC. On day 10 PC, morbidity occurred only in the unsupplemented-challenged Group C (2 birds). By day 15 PC, morbidity increased to 7 birds in the supplemented-challenged Group B and 9 birds in Group C (Table 6), consistent with peak clinical manifestation of *Eimeria* infection. On day 20 PC, morbidity was recorded in the

unsupplemented-unchallenged Group A (2 birds) and supplemented-unchallenged Group D (1 bird), while it declined to 2 birds in group B and 3 in group C (Table 6), suggesting recovery in the challenged birds. On day 25 PC, morbidity was observed only in group A (3 birds). From day 30 to 35 PC, no morbidity occurred in any group. No mortality was recorded throughout the study, indicating that the infection, though clinically evident, was non-lethal under the experimental conditions.

Lesion scores differed significantly among treatment groups (Kruskal-Wallis, $p < 0.05$). The unsupplemented-challenged group (C) had the highest cumulative intestinal lesion score, followed by the supplemented-challenged group (B), while groups A and D recorded the lowest scores (Table 7) (Dunn's post-hoc test, $p < 0.05$). Gross examination of

the jejunum revealed mild petechial haemorrhages in Group A birds, enteritis evident from the serosal surface in Group B, and frank blood upon opening the jejunal serosa in Group C (Figure 2). The caeca of Group C contained blood in unopened segments; caecal cores were present in Group B, whereas Group D birds exhibited normal caecal architecture (Figure 2). Histopathology of the jejunum showed necrosis, inflammatory cell infiltration, villous loss, and mucosal haemorrhages in Groups B and C birds on days 10 and 25 post-challenge (PC), while Groups A and D birds exhibited normal histology. By day 35 PC, all groups showed moderate inflammatory cell infiltration and mucosal congestion in both the jejunum and caeca (Figure 3).

Table 4. Body weights gain (g) of broiler chickens fed diets supplemented with *Saccharomyces cerevisiae* (SC) and challenged with mixed *Eimeria* oocysts infection.

Days post infection (days)	Group A (Unsupplemented unchallenged)	Group B (Supplemented challenged)	Group C (Unsupplemented challenged)	Group D (Supplemented unchallenged)
Day 5	216.70 ^a (7.77)	132.95 ^b (18.56)	127.33 ^b (7.98)	265.00 ^c (11.60)
Day 10	555.44 ^a (20.60)	325.39 ^b (19.18)	323.44 ^b (19.77)	653.11 ^c (1.01)
Day 15	900.83 ^a (46.98)	538.84 ^b (37.80)	528.11 ^b (8.40)	928.61 ^a (18.86)
Day 20	1267.66 ^a (148.93)	924.16 ^b (77.33)	747.94 ^b (66.02)	1291.39 ^a (98.98)
Day 25	1685.21 ^a (175.89)	1173.39 ^{a,b} (167.77)	754.27 ^b (329.18)	1715.61 ^a (126.47)
Day 30	1943.34 ^{a,b} (180.39)	1476.33 ^{a,b} (213.86)	1371.17 ^b (96.81)	1958.67 ^a (191.67)
Day 35	2027.10 ^{a,c} (74.03)	1697.72 ^b (120.62)	1535.55 ^b (143.10)	2216.34 ^a (114.96)

Values are presented as means, with SEM in brackets; Mean values bearing different superscripts in the same row differ significantly ($p < 0.05$).

Table 5. Feed conversion ratio (FCR) of broiler chickens fed diets supplemented with *Saccharomyces cerevisiae* (SC) and challenged with mixed *Eimeria* oocysts infection.

Days post infection (days)	Group A (Unsupplemented unchallenged)	Group B (Supplemented challenged)	Group C (Unsupplemented challenged)	Group D (Supplemented unchallenged)
Day 5	2.26 ^a (0.19)	3.04 ^b (0.31)	3.02 ^b (0.10)	2.08 ^a (0.09)
Day 10	2.08 ^a (0.04)	2.93 ^b (0.16)	2.94 ^b (0.06)	1.90 ^a (0.03)
Day 15	2.13 ^a (0.03)	2.89 ^b (0.20)	2.88 ^b (0.07)	2.06 ^a (0.04)
Day 20	2.21 ^a (0.18)	2.44 ^{a b} (0.16)	2.93 ^b (0.16)	2.16 ^a (0.16)
Day 25	2.18 (0.13)	2.71 (0.42)	2.72 (0.13)	2.13 (0.12)
Day 30	2.38 (0.12)	2.70 (0.32)	2.79 (0.16)	2.35 (0.17)
Day 35	2.78 ^{a c} (0.13)	2.78 ^{a c} (0.17)	3.08 ^{a b} (0.14)	2.43 ^c (0.06)

Values are presented as means, with SEM in brackets; Mean values bearing different superscripts in the same row differ significantly ($p < 0.05$).

Table 6. Morbidity pattern in broiler chickens fed diets supplemented with *Saccharomyces cerevisiae* (SC) and challenged with mixed *Eimeria* oocysts infection.

Days post infections (days)	Group A (Unsupplemented unchallenged)	Group B (Supplemented challenged)	Group C (Unsupplemented challenged)	Group D (Supplemented unchallenged)
Day 0	0/18	0/18	0/18	0/18
Day 5	0/18	0/18	0/18	0/18
Day 10	0/18	0/18	2/18	0/18
Day 15	0/18	7/18 *	9/18 *	0/18
Day 20	2/18	2/18	3/18	1/18
Day 25	3/18	0/18	0/18	0/18
Day 30	0/18	0/18	0/18	0/18
Day 35	0/18	0/18	0/18	0/18

Values are presented as number exhibiting clinical signs and lesions of coccidiosis / total number in the group. * Asterisk indicates day and group with the highest morbidity.

Table 7. Lesion scores of different parts of the intestines of broiler chickens fed diets supplemented with *Saccharomyces cerevisiae* (SC) and challenged with mixed *Eimeria* oocysts infection.

Parts of the intestines	Lesion scores *											
	Day 10 post infection				Day 25 post infection				Day 35 post infection			
Groups #	A	B	C	D	A	B	C	D	A	B	C	D
Duodenum	0	2	3	0	1	3	3	1	0	0	2	0
Jejunum	0	2	4	0	1	2	4	0	2	1	2	1
Ileum	0	0	2	0	1	1	2	1	0	0	1	1
Caeca	0	4	0	0	1	0	4	0	0	0	1	0
Colon	0	2	1	0	1	1	1	1	0	0	1	1
Totals	0	10	10	0	5	7	14	3	2	1	7	3

* Lesion scores were based on Shirley and Harvey score pattern (Shirley and Harvey, 1996).

Groups: A – Unsupplemented unchallenged group; B – Supplemented challenged group; C – Unsupplemented challenged; D – Supplemented unchallenged.

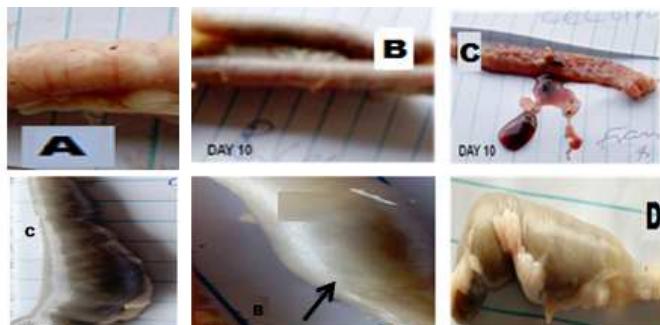


Figure 2. Photographs showing gross appearance of portions of the intestine of broiler chickens given dietary supplements of *Saccharomyces cerevisiae* and infected with mixed *Eimeria* oocysts: A (top left) – The jejunum of Group A with mild petechial haemorrhages; B (top middle) - Enteritis visible from the serosal surface Group B birds; C (top right) – Frank blood upon opening the jejunum of Group C birds.; C (down left) - Caecum of Group C birds with blood in unopened segments; B (down middle) – Caecal cores in Group B birds; and D (down right) – Caecum of Group D birds with normal architecture.

[Group A – Unsupplemented-unchallenged; Group B – Supplemented-challenged; Group C - Unsupplemented-challenged; and Group D – Supplemented-unchallenged]

Discussion

Morbidity occurred in both unchallenged and challenged birds, but it was earlier and more severe in challenged groups, which showed significantly worse disease indicators (Graham et al., 2023). Coccidiosis remains highly transmissible and ubiquitous despite

preventive measures (Quiroz-Castañeda and Dantán-González, 2015). Reports also suggest that unchallenged birds may sometimes become infected, likely due to the proclivity of *Eimeria* species and environmental contamination (Macdonald et al., 2017). Supplemented-challenged birds had lower morbidity than unsupplemented-challenged

counterparts, supporting evidence that *S. cerevisiae* can bind pathogens and toxins, potentially including *Eimeria*, thereby reducing morbidity and mortality (Mahmoud and Igarashi, 2012).

Feed intake, body weight, and weight gain were highest in unsupplemented-unchallenged and supplemented-unchallenged groups, with the latter showing a non-significant numerical advantage. Yeast supplementation has previously been shown to enhance broiler performance and intestinal

immune responses under coccidial challenge (Shanmugasundaram et al., 2013). The ability of *S. cerevisiae* supplementation to improve digestive efficiency, modulate gut microbiota, enhance immune function and reduce oxidative stress likely contributed to the improved performance and reduced morbidity seen in supplemented groups (Kabir, 2009; Kumar et al., 2023). Similar protective effects have been reported where *S. cerevisiae* β -glucan reduced coccidial lesion severity in challenged broilers (Cox et al., 2010).

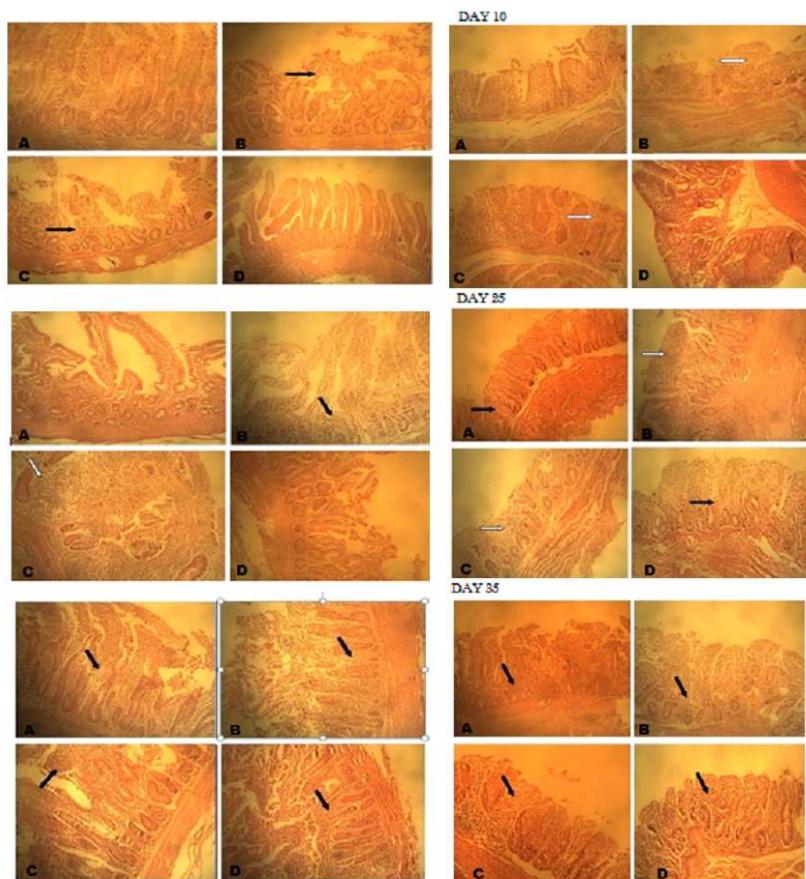


Figure 3. Photomicrographs of portions of the intestines of broiler chickens that were given dietary supplements of *Saccharomyces cerevisiae* and infected with mixed *Eimeria* oocysts (jejunum on the left and caeca on the right): Top two sets of microphotographs shows the jejunum of Groups B and C birds with necrosis, inflammatory cell infiltration, villous loss and mucosal haemorrhages on days 10 and 25 post-infection, with Groups A and D birds having with normal histology on the same days. Lower set of microphotographs shows and all groups with minimal inflammatory cell infiltration and mucosal congestion in the jejunum and caeca on day 35 post-infection. [H & E, $\times 100$]

[Group A – Unsupplemented-unchallenged; Group B – Supplemented-challenged; Group C - Unsupplemented-challenged; and Group D – Supplemented-unchallenged]

Lesions were detected in the duodenum, jejunum, ileum, and ceca of both supplemented-challenged and unsupplemented-challenged birds, with eventual spread to unchallenged groups. Balestrin et al. (2022) likewise reported lesion distribution across the duodenum (87.5%), jejunum (70.3%), ileum (18.8%), and cecum (46.9%) in challenged broilers. These findings confirm that mixed *Eimeria* inocula affect all intestinal regions (Graham et al., 2023) through life-cycle-mediated tissue damage (Gerhold, 2023).

In this study, jejunal and caecal lesion scores were significantly higher ($p < 0.05$) in challenged than in unchallenged birds, with supplementation tending to reduce severity. Giannenas et al. (2023) reported similar trends, showing that yeast fractions administered with anti-coccidials mitigated but did not eliminate lesion severity. Although *S. cerevisiae* supports intestinal health through increased villus height and reduced epithelial loss, it does not directly treat *Eimeria* infection (Luquetti et al., 2012; Adhikari et al., 2020).

Gross lesions in the jejunum and caecum included petechial haemorrhages on the serosa, catarrhal mucosal inflammation, and frank or clotted caecal blood. Such features are consistent with classical coccidiosis necropsy findings of mucosal congestion, oedema, and haemorrhage (Andrews, 2022). Histopathological examination revealed severe villous loss, mucosal haemorrhage, and inflammatory infiltration at early stages, followed by partial resolution characterized by moderate caecal inflammation, in contrast with normal histology in unchallenged birds. These changes typify *Eimeria*-induced villous atrophy, haemorrhage, inflammation, and epithelial necrosis (Long, 1993; Gerhold, 2023).

Conclusion: Mixed sporulated *Eimeria* oocysts (1×10^4) successfully induced coccidiosis in both supplemented and unsupplemented broilers, causing intestinal tissue damage and

reduced growth. However, weight gain was significantly higher ($p < 0.05$) in unchallenged groups, and supplementation with *S. cerevisiae* lowered morbidity, clinical signs, and lesion severity, though it did not provide complete protection. Dietary inclusion of this probiotic is therefore recommended to enhance growth performance, improve feed conversion, and support coccidiosis control.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the research work and publication of this article.

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