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Efficacy of *Saccharomyces boulardii* in mitigating necrotic enteritis induced by *Clostridium perfringens* in broiler chickens

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Abstract

Necrotic enteritis, caused by *Clostridium perfringens*, remains one of the most economically important enteric diseases affecting commercial broiler production worldwide. This study evaluated the efficacy of *Saccharomyces boulardii* as a non-antibiotic alternative for mitigating experimentally induced necrotic enteritis in broiler chickens. A total of 100 day-old Vencobb broiler chicks were randomly allocated into five groups (n = 20 per group). The groups comprised an unchallenged control (T₁), challenged control (T₂), challenged birds supplemented with enramycin (0.1 g/kg feed; T₃), challenged birds receiving prophylactic *Saccharomyces boulardii* (1×10^8 CFU/kg feed from day 1; T₄), and challenged birds receiving therapeutic *Saccharomyces boulardii* after the onset of clinical signs (T₅). Necrotic enteritis was induced by oral administration of a tenfold dose of Livacox® Q coccidia vaccine on the 13th day, followed by oral challenge with *Clostridium perfringens* (1×10^7 CFU/mL) for three consecutive days from the 17th day. Growth performance, serum biochemical profile, gross and microscopic lesions, intestinal morphometry, and intestinal *Clostridium perfringens* colonization were evaluated. Challenged control birds (T₂) showed significantly reduced growth performance, impaired feed efficiency, decreased serum total protein and globulin concentrations, elevated hepatic enzyme activities, severe intestinal lesions, villus atrophy, crypt hyperplasia, and increased intestinal bacterial load ($P < 0.05$). Prophylactic supplementation with *Saccharomyces boulardii* (T₄) markedly improved final body weight (2230.15 g), body weight gain (919.40 g), and feed conversion ratio (1.18), restoring performance to levels comparable to the negative control. These birds also exhibited preservation of jejunal villus architecture, reflected by greater villus height (1712.00 μ m on the 38th day) and an improved villus height-to-crypt depth ratio, together with reduced alanine aminotransferase (27.83 IU/L) and aspartate aminotransferase (175.65 IU/L) activities. Intestinal *Clostridium perfringens* counts declined from $6.15 \log_{10}$ CFU/g in challenged controls to $4.14 \log_{10}$ CFU/g following prophylactic *Saccharomyces boulardii* supplementation. Therapeutic administration of *Saccharomyces boulardii* also improved productive performance and intestinal health, although the response was less pronounced than that observed with prophylactic supplementation. The protective effects of prophylactic *Saccharomyces boulardii* were comparable to those achieved with enramycin treatment. These findings indicate that dietary supplementation with *Saccharomyces boulardii*, particularly when administered prophylactically, effectively mitigates the detrimental effects of *Clostridium perfringens*-induced necrotic enteritis and may represent a promising non-antibiotic strategy for maintaining intestinal health and productivity in broiler chickens.

Keywords: *Saccharomyces boulardii*, *Clostridium perfringens*, necrotic enteritis, broiler chickens, probiotic, intestinal morphology

Introduction

The global poultry industry plays a critical role in food and nutrition security by supplying affordable, high-quality animal protein to a growing population. Rapid intensification of poultry production through genetic improvement, balanced nutrition, enhanced biosecurity, and efficient management has substantially increased productivity worldwide. India has emerged as a major contributor, ranking as the world's second-largest egg producer and fourth-largest broiler meat producer (Honyal *et al.*, 2025; DAHD, 2025) [1, 2]. Despite these advances, enteric diseases remain a major constraint to broiler production, adversely affecting growth performance, animal welfare, and economic returns.

Necrotic enteritis (NE), caused by the Gram-positive, spore-forming, anaerobic bacterium *Clostridium perfringens*, is one of the most economically important enteric diseases in poultry. The disease occurs in both clinical and subclinical forms, resulting in mortality, impaired growth, poor feed conversion ratio (FCR), intestinal damage, and increased carcass condemnation (Shojadoost *et al.*, 2012; Fathima *et al.*, 2022) [3, 4]. Broiler chickens between 2 and 6 weeks of age are particularly susceptible (Timbermont *et al.*, 2011) [5]. Although *C. perfringens* is a normal inhabitant of the intestinal microbiota, factors such as coccidial infection, high-protein diets, poor litter quality, and high stocking density promote bacterial overgrowth and toxin production (McDevitt *et al.*, 2006) [6]. Among the toxins produced, NetB has been recognized as a major virulence factor associated with avian NE (Keyburn *et al.*, 2008) [7].

For many years, necrotic enteritis was effectively controlled through the routine use of antimicrobial growth promoters (AGPs). However, increasing concerns regarding antimicrobial resistance (AMR), food safety, and consumer preference have led to restrictions on AGP use, creating an urgent need for sustainable alternatives to control *C. perfringens* infections (Diaz *et al.*, 2019; Abd El-Hack *et al.*, 2020) [8, 9]. Consequently, nutritional strategies incorporating probiotics, prebiotics, organic acids, phytogenic compounds, and exogenous enzymes have gained considerable attention for maintaining intestinal health and improving disease resistance (Moore, 2016; Bansal *et al.*, 2021; Moore, 2024) [10, 11, 12].

Among these alternatives, probiotics have shown considerable promise in reducing enteric diseases by competitively excluding pathogens, producing antimicrobial metabolites, enhancing intestinal barrier function, and modulating host immune responses (Bermudez-Brito *et al.*, 2012; Liao & Nyachoti, 2017; Yaqoob *et al.*, 2022) [13, 14, 15]. Various bacterial and yeast probiotics have demonstrated beneficial effects against *C. perfringens* infection and improved productive performance in broiler chickens (Jha *et al.*, 2020; Emami *et al.*, 2021) [16, 17].

Saccharomyces boulardii, a non-pathogenic probiotic yeast, has attracted considerable interest because of its unique biological properties and established safety profile. Unlike many bacterial probiotics, *S. boulardii* secretes proteolytic enzymes capable of degrading bacterial toxins, enhances intestinal barrier integrity, and modulates mucosal immune responses, thereby protecting the intestinal epithelium from pathogen-induced injury (Mumy *et al.*, 2008; Pothoulakis, 2009; Rajput *et al.*, 2013) [18, 19, 20]. Dietary supplementation with *S. boulardii* has also been associated with improved growth performance, feed efficiency, intestinal histomorphology, and overall gut health in poultry (Yaqoob *et al.*, 2022) [15].

Despite these promising biological properties, studies evaluating the efficacy of *S. boulardii* against experimentally induced *Clostridium perfringens*-associated necrotic enteritis in broiler chickens remain limited. Most available reports have focused on *Saccharomyces cerevisiae* or mixed probiotic preparations, leaving limited evidence regarding the protective efficacy of *S. boulardii* under controlled challenge conditions (Caly *et al.*, 2015) [21]. Therefore, the present study was undertaken to evaluate the efficacy of prophylactic and therapeutic supplementation with *Saccharomyces boulardii* in mitigating experimentally induced necrotic enteritis caused by *Clostridium perfringens*

in broiler chickens by assessing growth performance, serum biochemical profile, intestinal pathology, intestinal morphometry, and bacterial colonization.

Materials and Methods

Experimental birds and management

A total of 100 healthy day-old Vencobb broiler chicks and reared for 38 days under standard management conditions. Birds were housed in separate floor pens bedded with rice husk litter and provided *ad libitum* access to feed and drinking water throughout the experimental period. Routine vaccination against Newcastle disease and infectious bursal disease was carried out according to standard poultry management practices.

Experimental design and induction of necrotic enteritis

The birds were randomly allocated into five experimental groups (T₁–T₅), each consisting of 20 birds. Group T₁ served as the unchallenged control and received only the basal diet. Birds in groups T₂, T₃, T₄, and T₅ were subjected to experimental induction of necrotic enteritis.

To predispose birds to necrotic enteritis, a tenfold dose of Livacox® Q coccidia vaccine was administered orally on day 13. On day 16, birds were subjected to overnight feed withdrawal as described by Gholamiandehkordi *et al.* (2007) [22]. Experimental infection was then induced by oral administration of *Clostridium perfringens* Type A culture (1×10^7 CFU/mL) at a dose of 1 mL per bird for three consecutive days from day 17.

The treatment groups were as follows

- T₁: Basal diet (unchallenged control)
- T₂: Challenged control (no supplementation)
- T₃: Challenged birds supplemented with enramycin (0.1 g/kg feed) from day 1
- T₄: Challenged birds supplemented with *Saccharomyces boulardii* (1×10^8 CFU/kg feed) from day 1 (prophylactic treatment)
- T₅: Challenged birds supplemented with *Saccharomyces boulardii* (1×10^8 CFU/kg feed) following the appearance of clinical signs (therapeutic treatment)

Preparation of *Clostridium perfringens* inoculum

Clostridium perfringens (MTCC 450) was obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The culture was revived in Reinforced Clostridial Medium (RCM) broth and incubated anaerobically at 37°C for 24 h. Bacterial density was standardized using McFarland standard No. 2 and confirmed spectrophotometrically at 600 nm ($OD_{600} = 0.756$). The culture was subsequently diluted with sterile phosphate-buffered saline to obtain the desired challenge concentration of 1×10^7 CFU/mL (Rathnapraba, 2021) [23].

Probiotic and antibiotic supplementation

The probiotic yeast *Saccharomyces boulardii* was incorporated into the feed at a concentration of 1×10^8 CFU/kg feed. Enramycin was supplemented at 0.1 g/kg feed according to the experimental design. Prophylactic supplementation was initiated from day 1, whereas therapeutic supplementation commenced following the onset of clinical signs associated with necrotic enteritis.

Parameters studied

Clinical observations and growth performance

All birds were monitored twice daily throughout the experimental period for the occurrence of clinical signs, morbidity, and mortality. Body weight and feed intake were recorded weekly. Body weight gain and feed conversion ratio (FCR) were calculated from the weekly data.

Serum biochemical analysis

Blood samples were collected from the heart and wing vein on 0th, 7th, 14th and 21st day post-infection. Serum was separated and stored at -20°C until analysis. Serum total protein, albumin, globulin, albumin-to-globulin (A/G) ratio, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were estimated using a fully automated biochemical analyzer following the manufacturer's instructions.

Gross and histopathological examination

Necropsy examinations were performed on all birds that died during the experiment as well as on representative birds sacrificed at day 24 (7th day post-infection) and day 38 (21st day post-infection). Gross lesions were recorded in the liver, spleen, kidneys, bursa of Fabricius, and intestinal tract (duodenum, jejunum, and ileum).

For histopathological examination, tissue samples from the duodenum, jejunum, ileum, liver, spleen, kidneys, and bursa of Fabricius were collected in 10% neutral buffered formalin. Following routine tissue processing, paraffin-embedded sections (4–6 µm) were prepared and stained with hematoxylin and eosin according to the method described by Luna (1968) [24].

Intestinal morphometry

Jejunum and ileal tissue sections collected on day 24 and day 38 were subjected to morphometric evaluation. Villus height, crypt depth, and villus height-to-crypt depth ratio (VH:CD) were measured using an ocular micrometer to assess intestinal integrity and absorptive capacity.

Re-isolation and enumeration of *Clostridium perfringens*

Intestinal contents collected from the ileo-caecal region on day 24 and day 38 were used for re-isolation and enumeration of *C. perfringens*. Samples were enriched in Reinforced Clostridial Broth under anaerobic conditions and subsequently cultured on Tryptose Sulfite Cycloserine (TSC) agar and Sulfite Polymyxin Sulfadiazine (SPS) agar. Suspected colonies were confirmed by Gram staining, lecithinase activity on egg yolk agar, and stormy fermentation in litmus milk.

For quantitative enumeration, one gram of intestinal content was serially diluted in sterile phosphate-buffered saline and plated on TSC agar. After anaerobic incubation at 37°C for 24 h, characteristic black colonies were counted and expressed as log₁₀ CFU/g of intestinal content.

Statistical analysis

All data were analyzed using a Completely Randomized Design (CRD) according to the procedures described by Snedecor and Cochran (1989) [25]. Results were expressed as mean ± standard error (SE). Differences among treatment groups were considered statistically significant at $P < 0.05$.

Results

Growth Performance Parameters

Feed intake

Feed intake did not differ among treatment groups during the first week ($P > 0.05$) (Table 1), whereas significant differences were observed from the second week onward ($P < 0.05$) (Table 1). During week 2, birds receiving prophylactic *Saccharomyces boulardii* (T₄) exhibited the highest feed intake (297.60 ± 0.57 g/bird), followed by the enramycin-treated group (T₃; 289.00 ± 0.41 g/bird), whereas T₁, T₂, and T₅ showed comparable feed intake (281.50 – 282.10 g/bird). Following *Clostridium perfringens* challenge, feed intake declined in all challenged groups during week 3, with the greatest reduction observed in the therapeutic probiotic group (T₅; 595.00 ± 0.41 g/bird) and the infected untreated group (T₂; 599.75 ± 0.75 g/bird). The negative control (T₁) maintained the highest feed intake during the challenge period (611.80 ± 0.27 g/bird). During the final week, T₂ recorded the lowest feed intake (1072.13 ± 0.63 g/bird), whereas T₃ maintained the highest intake among the challenged groups (1095.25 ± 0.49 g/bird), followed by T₄ (1083.88 ± 0.83 g/bird) and T₅ (1081.06 ± 0.54 g/bird).

Body weight

Body weight did not differ among treatment groups during the first week ($P > 0.05$), whereas significant differences were observed from week 2 onward ($P < 0.05$) (Table 1). During week 2, T₃ recorded the highest body weight (414.50 ± 1.26 g), whereas T₅ showed the lowest value (394.50 ± 1.26 g). Following bacterial challenge, body weight decreased markedly in the infected untreated group. During week 3, T₂ recorded the lowest body weight (738.00 ± 2.55 g), while T₁, T₃, T₄, and T₅ remained statistically comparable (813.75 – 823.00 g). This trend continued during weeks 4 and 5, with T₂ consistently exhibiting the lowest body weight (1215.00 ± 1.08 and 1989.00 ± 4.53 g, respectively). By the end of the experiment, birds in T₄ achieved a final body weight (2230.15 ± 3.22 g) comparable to the negative control (2233.45 ± 2.11 g), whereas T₃ and T₅ recorded intermediate values.

Body weight gain

Weekly body weight gain did not differ among treatment groups during the first week but varied significantly from week 2 onward ($P < 0.05$) (Table 1). Before bacterial challenge, T₃ exhibited the highest body weight gain during week 2 (260.50 ± 0.50 g), whereas T₅ recorded the lowest gain (244.25 ± 0.46 g). Following *C. perfringens* challenge, birds in T₂ demonstrated a marked reduction in body weight gain, recording the lowest value during week 3 (328.90 ± 3.07 g), compared with T₁ (421.23 ± 4.07 g), T₄ (415.35 ± 3.90 g), and T₅ (419.25 ± 5.28 g). During week 4, T₁ maintained the highest body weight gain (547.00 ± 4.40 g), whereas T₂ remained significantly lower (477.00 ± 2.38 g). By week 5, T₄ (919.40 ± 4.79 g) and T₅ (914.88 ± 4.36 g) recorded the highest body weight gains and did not differ significantly from each other, whereas T₂ continued to show the lowest body weight gain (774.00 ± 5.05 g).

Feed conversion ratio: Feed conversion ratio (FCR) did not differ among treatment groups during the first week ($P > 0.05$) but differed significantly during weeks 2–5 ($P < 0.05$) (Table 1). During week 2, T₄ recorded the highest FCR (1.18 ± 0.01), whereas T₂ and T₃ showed the lowest

values (1.10–1.11). Following *C. perfringens* challenge, FCR increased markedly in the infected untreated group, reaching 1.82 ± 0.02 during week 3 and remaining the highest during the final week (1.39 ± 0.01). In contrast,

birds receiving prophylactic *S. bouhardii* (T₄) and therapeutic supplementation (T₅) recorded the lowest FCR during week 5 (1.18 ± 0.01), followed by T₃ (1.22 ± 0.01).

Table 1: Mean ($\pm$ SE) values of growth performance parameters of experimental birds at different intervals of study

Parameter	Timepoint	T ₁	T ₂	T ₃	T ₄	T ₅
Feed intake (g/bird)	Wk 1 (pre-challenge)	126.50 $\pm$ 0.65	126.35 $\pm$ 0.77	127.03 $\pm$ 0.52	126.48 $\pm$ 0.53	126.25 $\pm$ 0.54
	Wk 3 (challenge)	611.80 ^a $\pm$ 0.27	599.75 ^c $\pm$ 0.75	597.10 ^d $\pm$ 0.70	604.00 ^b $\pm$ 0.41	595.00 ^c $\pm$ 0.41
	Wk 5 (final)	1113.25 ^a $\pm$ 0.78	1072.13 ^c $\pm$ 0.63	1095.25 ^b $\pm$ 0.49	1083.88 ^c $\pm$ 0.83	1081.06 ^d $\pm$ 0.54
Body weight (g)	Wk 1 (pre-challenge)	152.88 $\pm$ 0.63	152.15 $\pm$ 0.91	154.00 $\pm$ 0.82	152.23 $\pm$ 1.09	150.25 $\pm$ 0.85
	Wk 3 (challenge)	823.00 ^a $\pm$ 3.87	738.00 ^b $\pm$ 2.55	819.50 ^a $\pm$ 4.03	819.83 ^a $\pm$ 3.09	813.75 ^a $\pm$ 4.87
	Wk 5 (final)	2233.45 ^a $\pm$ 2.11	1989.00 ^c $\pm$ 4.53	2217.48 ^b $\pm$ 3.12	2230.15 ^a $\pm$ 3.22	2209.60 ^b $\pm$ 3.37
Body weight gain (g/bird)	Wk 1 (pre-challenge)	110.88 $\pm$ 0.63	110.15 $\pm$ 0.87	112.28 $\pm$ 0.86	110.75 $\pm$ 0.85	108.93 $\pm$ 0.60
	Wk 3 (challenge)	421.23 ^a $\pm$ 4.07	328.90 ^c $\pm$ 3.07	405.00 ^b $\pm$ 3.87	415.35 ^{ab} $\pm$ 3.90	419.25 ^a $\pm$ 5.28
	Wk 5 (final)	863.45 ^c $\pm$ 3.44	774.00 ^d $\pm$ 5.05	899.98 ^b $\pm$ 5.97	919.40 ^a $\pm$ 4.79	914.88 ^a $\pm$ 4.36
FCR	Wk 1 (pre-challenge)	1.14 $\pm$ 0.01	1.15 $\pm$ 0.00	1.13 $\pm$ 0.01	1.14 $\pm$ 0.01	1.16 $\pm$ 0.01
	Wk 3 (challenge)	1.45 ^{bc} $\pm$ 0.01	1.82 ^a $\pm$ 0.02	1.47 ^b $\pm$ 0.01	1.45 ^{bc} $\pm$ 0.01	1.42 ^c $\pm$ 0.02
	Wk 5 (final)	1.29 ^b $\pm$ 0.01	1.39 ^a $\pm$ 0.01	1.22 ^c $\pm$ 0.01	1.18 ^d $\pm$ 0.01	1.18 ^d $\pm$ 0.01

Values are presented as mean $\pm$ SE. Means within a column bearing different superscripts differ significantly ($P < 0.05$). (Wk- Week)

Serum Biochemical Parameters

Serum total protein concentrations did not differ among treatment groups before bacterial challenge (0th day; $P > 0.05$), whereas significant differences were observed on 7th, 14th, and 21st day post-infection ($P < 0.05$) (Table 2). On 7th day, the infected untreated group (T₂) recorded the lowest serum total protein concentration (2.95 ± 0.06 g/dL), which was comparable to T₅ (2.99 ± 0.03 g/dL) but significantly lower than T₁ (4.25 ± 0.08 g/dL), T₃ (4.00 ± 0.02 g/dL), and T₄ (3.96 ± 0.10 g/dL). On 14th day, T₂ (3.32 ± 0.05 g/dL) remained significantly lower than all other groups, whereas T₅ (3.59 ± 0.07 g/dL) recorded intermediate values. By 21st day, serum total protein concentrations increased in all treatment groups; however, T₂ (3.60 ± 0.04 g/dL) remained significantly lower than T₁ (4.33 ± 0.08 g/dL), T₃ (4.19 ± 0.08 g/dL), T₄ (4.10 ± 0.02 g/dL), and T₅ (3.94 ± 0.07 g/dL).

Serum albumin concentrations did not differ significantly among treatment groups throughout the experimental period ($P > 0.05$). Although numerically lower albumin concentrations were recorded in T₂ and T₅ following bacterial challenge, the differences were not statistically significant at any sampling interval.

Serum globulin concentrations were comparable among treatment groups before infection ($P > 0.05$) but differed significantly on 7th, 14th, and 21st day post infection ($P < 0.05$) (Table 2). On 7th day, T₁ (2.07 ± 0.17 g/dL), T₃ (1.95 ± 0.11 g/dL), and T₄ (1.99 ± 0.19 g/dL) recorded significantly higher globulin concentrations than T₂ (1.31 ± 0.10 g/dL) and T₅ (1.11 ± 0.10 g/dL). A similar trend was observed on 14th day, with T₂ (1.47 ± 0.08 g/dL) and T₅ (1.62 ± 0.09 g/dL) recording significantly lower globulin concentrations than T₁, T₃, and T₄. By 21st day, T₁ (2.04 ± 0.13 g/dL) and T₃ (1.94 ± 0.19 g/dL) maintained significantly higher globulin concentrations than the remaining treatment groups, whereas T₂ (1.38 ± 0.19 g/dL) recorded the lowest value.

No significant differences were observed in the albumin-to-globulin (A/G) ratio throughout the experimental period ($P > 0.05$). The A/G ratio ranged from 1.09 ± 0.05 to 1.10 ± 0.17 before infection and from 1.04 ± 0.16 to 1.76 ± 0.23 ,

1.08 ± 0.13 to 1.28 ± 0.13 , and 1.15 ± 0.12 to 1.75 ± 0.35 on 7th, 14th, and 21st day post-infection, respectively.

Serum cholesterol concentrations were comparable among treatment groups before bacterial challenge ($P > 0.05$) but differed significantly on 7th, 14th, and 21st day post-infection ($P < 0.05$) (Table 2). On 7th day, T₂ recorded the lowest cholesterol concentration (101.98 ± 0.57 mg/dL), followed by T₅ (118.28 ± 0.38 mg/dL), T₄ (123.11 ± 0.65 mg/dL), T₃ (128.00 ± 0.76 mg/dL), and T₁ (134.75 ± 0.64 mg/dL). Similar differences were observed on day 14, with cholesterol concentrations ranging from 112.37 ± 0.73 mg/dL in T₂ to 141.00 ± 0.47 mg/dL in T₁. On 21st day, T₁ (145.30 ± 0.40 mg/dL) maintained the highest cholesterol concentration, whereas T₂ (131.16 ± 0.33 mg/dL) recorded the lowest value. No significant difference was observed between T₄ and T₅ on 21st day.

Serum alanine aminotransferase (ALT) activity did not differ among treatment groups before infection ($P > 0.05$) but differed significantly on 7th, 14th, and 21st day post-infection ($P < 0.05$) (Table 2). The infected untreated group (T₂) consistently recorded the highest ALT activity, with values of 52.83 ± 0.47 , 50.13 ± 0.50 , and 46.78 ± 0.49 IU/L on 7th, 14th, and 21st day, respectively. In contrast, T₁ recorded the lowest ALT activity, whereas T₃, T₄, and T₅ showed intermediate values throughout the post-infection period.

Serum aspartate aminotransferase (AST) activity was comparable among treatment groups before infection ($P > 0.05$) but differed significantly on 7th, 14th, and 21st day post-infection ($P < 0.05$) (Table 2). The infected untreated group recorded the highest AST activity, whereas T₁ maintained the lowest values throughout the study. T₃, T₄, and T₅ exhibited intermediate AST activities at all post-infection sampling intervals.

Serum creatinine concentrations did not differ among treatment groups before bacterial challenge ($P > 0.05$) but differed significantly following infection ($P < 0.05$) (Table 2). The infected untreated group recorded the highest creatinine concentrations, whereas T₁ maintained the lowest values throughout the post-infection period. Birds in T₃, T₄, and T₅ exhibited intermediate creatinine concentrations at all sampling intervals.

Table 2: Mean ($\pm$ SE) values of serum biochemical parameters of experimental birds at different intervals of study

Parameter	Day	T ₁	T ₂	T ₃	T ₄	T ₅
Total protein (g/dL)	0 th	4.06 $\pm$ 0.05	4.03 $\pm$ 0.08	4.06 $\pm$ 0.14	4.06 $\pm$ 0.07	4.05 $\pm$ 0.08
	7 th	4.25 ^a $\pm$ 0.08	2.95 ^c $\pm$ 0.06	4.00 ^b $\pm$ 0.02	3.96 ^b $\pm$ 0.10	2.99 ^c $\pm$ 0.03
	21 st	4.33 ^a $\pm$ 0.08	3.60 ^d $\pm$ 0.04	4.19 ^{ab} $\pm$ 0.08	4.10 ^{bc} $\pm$ 0.02	3.94 ^c $\pm$ 0.07
Globulin (g/dL)	0 th	1.95 $\pm$ 0.07	1.94 $\pm$ 0.08	1.97 $\pm$ 0.16	1.96 $\pm$ 0.13	1.97 $\pm$ 0.13
	7 th	2.07 ^a $\pm$ 0.17	1.31 ^b $\pm$ 0.10	1.95 ^a $\pm$ 0.11	1.99 ^a $\pm$ 0.19	1.11 ^b $\pm$ 0.10
	21 st	2.04 ^a $\pm$ 0.13	1.38 ^c $\pm$ 0.19	1.94 ^a $\pm$ 0.19	1.87 ^b $\pm$ 0.14	1.79 ^b $\pm$ 0.12
Cholesterol (mg/dL)	0 th	127.53 $\pm$ 1.37	125.88 $\pm$ 1.76	125.97 $\pm$ 1.37	125.00 $\pm$ 0.84	124.00 $\pm$ 0.89
	7 th	134.75 ^a $\pm$ 0.64	101.98 ^c $\pm$ 0.57	128.00 ^b $\pm$ 0.76	123.11 ^c $\pm$ 0.65	118.28 ^d $\pm$ 0.38
	21 st	145.30 ^a $\pm$ 0.40	131.16 ^d $\pm$ 0.33	142.99 ^b $\pm$ 0.21	138.00 ^c $\pm$ 0.56	137.79 ^c $\pm$ 0.81
ALT (IU/L)	0 th	26.08 $\pm$ 0.13	26.13 $\pm$ 0.11	26.00 $\pm$ 0.13	25.98 $\pm$ 0.09	26.03 $\pm$ 0.13
	7 th	27.38 ^c $\pm$ 0.19	52.83 ^a $\pm$ 0.47	40.85 ^c $\pm$ 0.48	31.53 ^d $\pm$ 0.25	45.13 ^b $\pm$ 0.53
	21 st	27.50 ^d $\pm$ 0.13	46.78 ^a $\pm$ 0.49	31.85 ^c $\pm$ 0.31	27.83 ^d $\pm$ 0.13	34.93 ^b $\pm$ 0.47
AST (IU/L)	0 th	174.93 $\pm$ 0.18	174.98 $\pm$ 0.17	174.78 $\pm$ 0.15	174.90 $\pm$ 0.13	174.93 $\pm$ 0.17
	7 th	175.73 ^c $\pm$ 0.21	203.85 ^a $\pm$ 0.47	192.28 ^c $\pm$ 0.39	182.40 ^d $\pm$ 0.29	196.58 ^b $\pm$ 0.36
	21 st	173.25 ^c $\pm$ 0.16	191.25 ^a $\pm$ 0.39	180.40 ^c $\pm$ 0.27	175.65 ^d $\pm$ 0.18	184.60 ^b $\pm$ 0.43
Creatinine (mg/dL)	0 th	0.85 $\pm$ 0.04	0.86 $\pm$ 0.03	0.85 $\pm$ 0.04	0.84 $\pm$ 0.06	0.87 $\pm$ 0.05
	7 th	0.89 ^{bc} $\pm$ 0.04	1.21 ^a $\pm$ 0.06	0.86 ^c $\pm$ 0.04	0.97 ^{bc} $\pm$ 0.06	1.06 ^{ab} $\pm$ 0.08
	21 st	0.95 ^b $\pm$ 0.04	1.40 ^a $\pm$ 0.05	0.97 ^b $\pm$ 0.05	1.05 ^b $\pm$ 0.03	1.26 ^a $\pm$ 0.07

Values are presented as mean $\pm$ SE. Means within a column bearing different superscripts differ significantly ($P < 0.05$).

Gross Pathological Findings

No gross lesions were observed in birds of the negative control group (T₁) throughout the experimental period. On the 24th day, birds in the infected untreated group (T₂) developed characteristic lesions of necrotic enteritis. The small intestine, particularly the jejunum and ileum, was markedly distended with gas and exhibited thin, friable intestinal walls. The duodenal and jejunal mucosa showed severe congestion, multifocal hemorrhages, ulceration, and adherent fibrinonecrotic pseudomembranes, producing the characteristic "turkey towel" appearance. Blood-tinged intestinal contents and fibrinous casts were frequently observed within the intestinal lumen. In addition to intestinal lesions, hepatomegaly (Fig 2) with moderate multifocal necrotic foci, enlarged congested kidneys, mild splenomegaly, and mild atrophy of the bursa of Fabricius were consistently observed.

Gross lesions were markedly less severe in the treated groups (Fig 1). Birds receiving enramycin (T₃) exhibited only mild intestinal distension with occasional focal hemorrhages, while prophylactic *Saccharomyces boulardii* supplementation (T₄) resulted in mild to moderate jejunal congestion and focal hemorrhages without pseudomembrane formation. Birds in the therapeutic group (T₅) exhibited lesions of intermediate severity characterized by intestinal ballooning, fibrinous exudation, mild hepatic enlargement with focal necrotic patches, mild renal congestion, and occasional splenic enlargement.

On the 38th day, gross lesions persisted in the infected untreated group (T₂) but with reduced severity. Residual lesions consisted of mild intestinal distension, mucosal congestion, occasional fibrinous casts within the terminal ileum, mild hepatomegaly with congestion, renal congestion, persistent mild splenomegaly, and bursal atrophy.

In contrast, birds in T₃ and T₄ exhibited near-normal gross morphology of the intestine, liver, kidneys, spleen, and bursa of Fabricius, with only minimal residual lesions. Birds receiving therapeutic *S. boulardii* (T₅) demonstrated partial resolution of intestinal and visceral lesions compared with those observed on the 24th day, although mild congestion and occasional intestinal friability persisted.

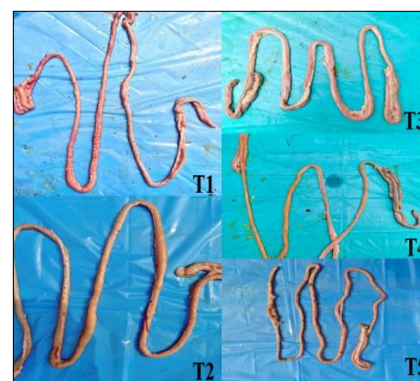


Fig 1: Comparative gross appearance of small intestine (duodenum, jejunum and ileum) on 24th day: T₁ (normal serosa); T₂ (marked distension, thickened walls and hemorrhages); T₃ (minimal distension with mild hemorrhages); T₄ (slight congestion with near-normal serosa); T₅ (moderate ballooning and congestion)

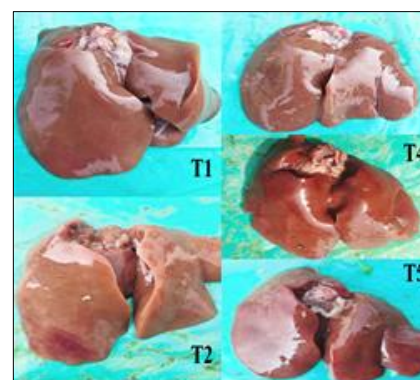


Fig 2: Comparative gross appearance of liver from different experimental groups on 24th day: T₁ (normal size and color); T₂ (hepatomegaly with congested parenchyma and necrotic foci); T₃ (mild congestion); T₄ (near-normal appearance); T₅ (mild enlargement with slight congestion)

Histopathological Findings

Histopathological examination was performed on the duodenum, jejunum, ileum, liver, kidneys, spleen, and bursa of Fabricius at 24th day and 38th day. No microscopic alterations were observed in tissues from the negative control group (T₁), which maintained normal histoarchitecture throughout the study.

In contrast, birds in the infected untreated group (T_2) developed characteristic microscopic lesions of necrotic enteritis, which were most severe on the 24th day. The duodenum exhibited moderate villus blunting and atrophy, focal villus fusion, epithelial desquamation, lamina propria edema, vascular congestion, and mild to moderate inflammatory mononuclear cell infiltration (Fig 3). Similar lesions were observed in the ileum but with comparatively lower severity. The jejunum exhibited the most pronounced pathological alterations (Fig 4), characterized by marked villus blunting and distortion, extensive epithelial desquamation, luminal necrotic debris, coagulative necrosis of villus tips, vascular congestion, and moderate to severe inflammatory cell infiltration in T_2 group.

Extraintestinal lesions were also evident in T_2 birds. Liver sections showed sinusoidal dilatation, vascular congestion, multifocal hemorrhages, and focal coagulative necrosis with mild distortion of hepatic architecture (Fig 7). Kidneys exhibited tubular epithelial degeneration with cytoplasmic vacuolation, hypercellular glomeruli, widened Bowman's space, and vascular congestion (Fig 9). The spleen showed marked sinusoidal congestion with expansion of the red pulp and mild to moderate lymphoid depletion of white pulp follicles (Fig 11), whereas the bursa of Fabricius exhibited moderate lymphoid depletion, interfollicular widening, and reduced follicular density (Fig 13).

Histopathological lesions were considerably less severe in the treated groups. Birds receiving enramycin (T_3) exhibited

relatively preserved intestinal architecture with only mild villus irregularity, minimal epithelial degeneration, and mild inflammatory cell infiltration (Fig 5), together with only slight hepatic, renal, splenic, and bursal alterations. Similarly, prophylactic *Saccharomyces boulardii* supplementation (T_4) maintained better intestinal architecture, with mild villus shortening, an intact epithelial lining, minimal inflammatory infiltration, and only mild changes in the visceral organs (Fig 6).

Liver sections from T_4 birds showed preserved lobular architecture with mild sinusoidal dilatation and focal periportal lymphoid aggregation (Fig 8), while kidney sections revealed preserved tubular and glomerular architecture with focal congestion of tubular epithelial cells (Fig 10). Likewise, splenic sections exhibited mild sinusoidal congestion with moderate lymphoid cellularity (Fig 12), and the bursa of Fabricius showed moderate lymphoid cellularity with mild interfollicular widening. Birds receiving therapeutic *S. boulardii* (T_5) initially exhibited lesions comparable to those of T_2 on the 24th day; however, by the 38th day, substantial restoration of intestinal architecture, reduced inflammatory infiltration, and marked improvement in hepatic, renal, splenic, and bursal lesions were observed. Residual microscopic lesions persisted in T_2 at the end of the experiment, whereas tissues from T_3 and T_4 appeared near normal and T_5 demonstrated partial histological recovery.

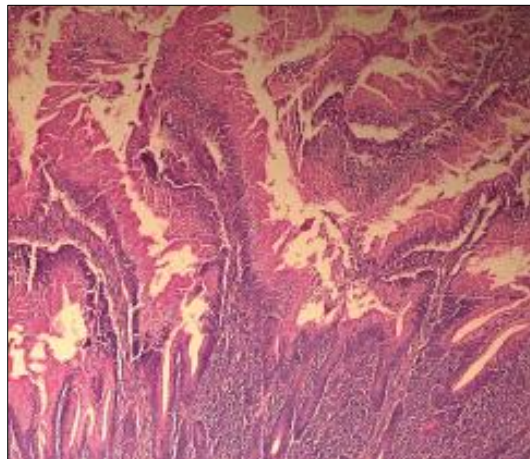


Fig 3: Duodenal section of T_2 group showing severe villous atrophy with blunting and marked MNC infiltration on 24th day (H&E, ×100)

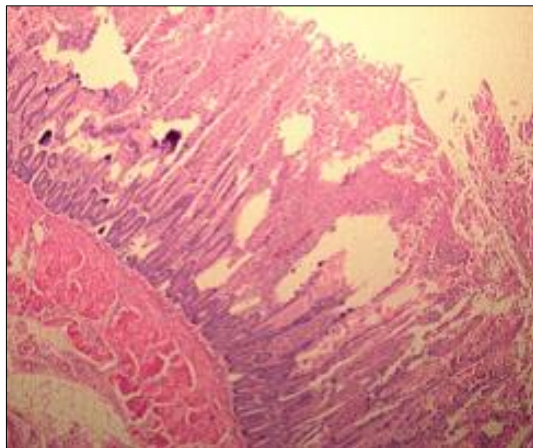


Fig 4: Jejunum section of T_2 group showing marked villus blunting and distortion with extensive epithelial desquamation and marked mucosal vascular congestion on 24th day (H&E, ×100)

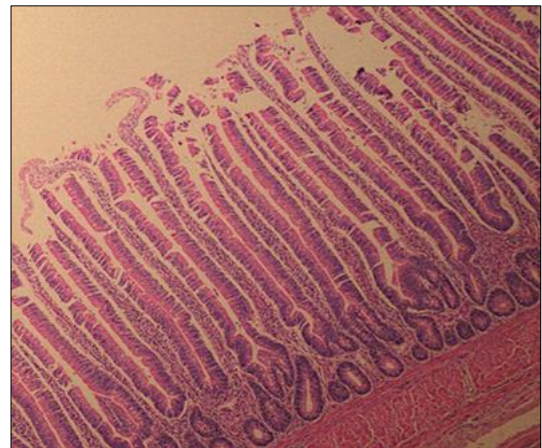


Fig 5: Jejunum section of T_3 group showing preserved villus architecture with mild villus tip irregularity on 24th day (H&E, ×100)

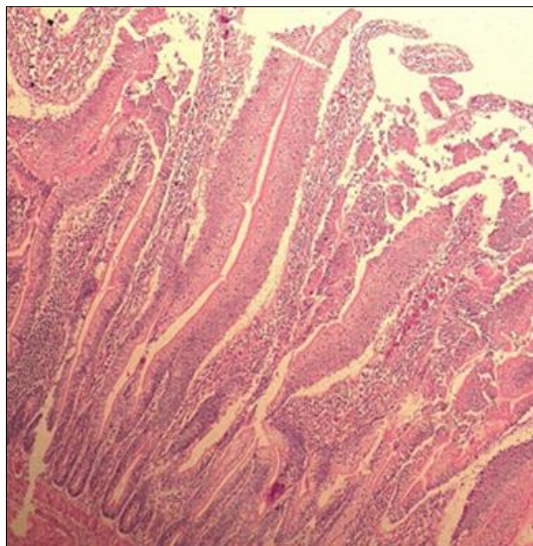


Fig 6: Note jejunum section of T₄ group showing mild villus irregularity with minimal inflammatory cell infiltration in the lamina propria and intact epithelial lining on 24th day (H&E, ×100)

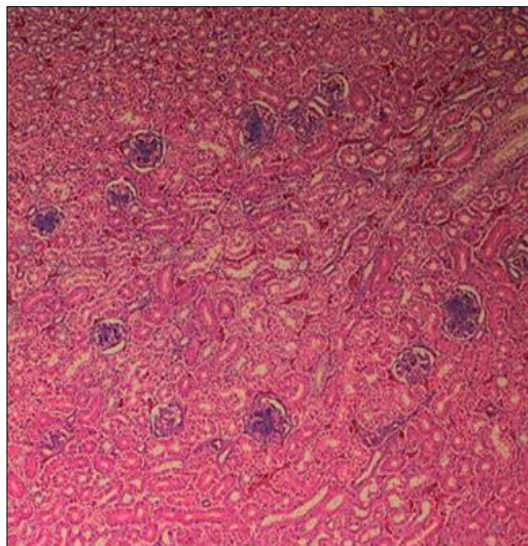


Fig 9: Kidney section of T₂ group showing tubular epithelial swelling, mildly hypercellular glomeruli with widened bowman's space and vascular congestion on 24th day (H&E, ×100)

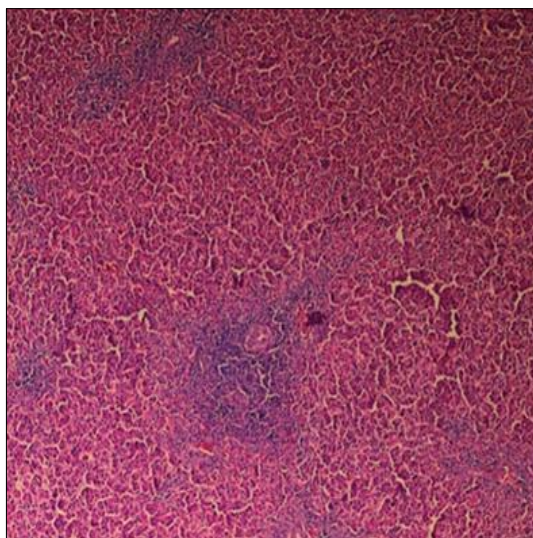


Fig 7: Liver section of T₂ group showing sinusoidal dilatation, multifocal hemorrhage and focal coagulative necrosis with inflammatory cell aggregates on 24th day (H&E, ×100)

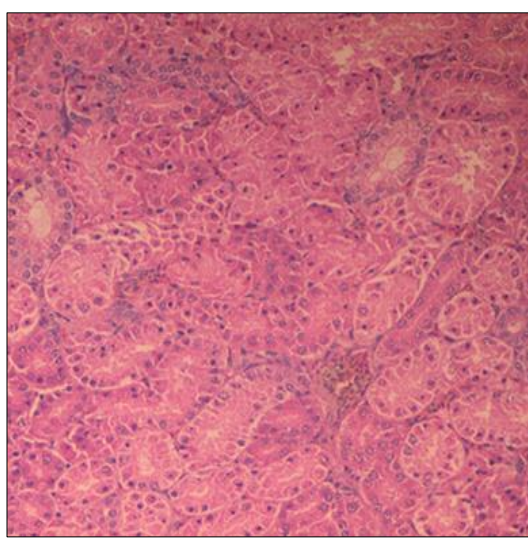


Fig 10: Kidney section of T₄ group showing preserved tubular and glomerular architecture with focal congestion of tubular epithelial cells on 24th day (H&E, ×400)

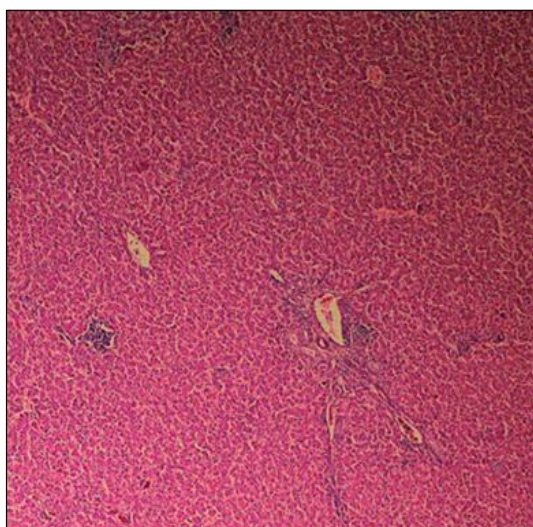


Fig 8: Liver section of T₄ group showing preserved lobular architecture with mild sinusoidal dilatation and focal periportal lymphoid aggregation on 24th day (H&E, ×100)

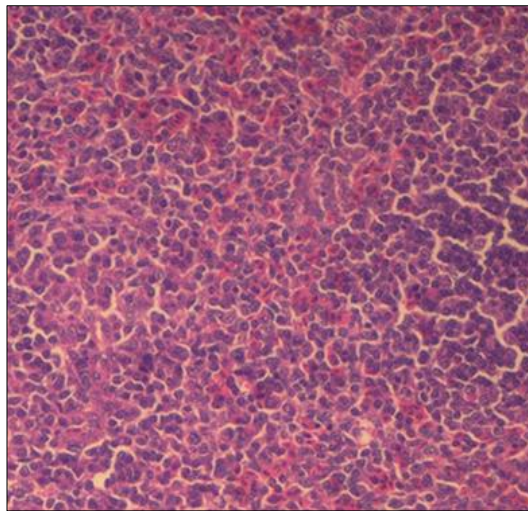


Fig 11: Spleen section of T₂ group showing marked sinusoidal congestion and mild to moderate lymphoid depletion in white pulp follicles on 24th day (H&E, ×400)

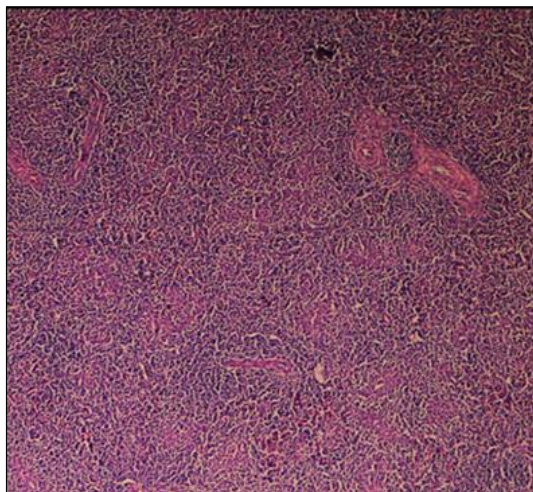


Fig 12: Spleen section of T4 group showing mild sinusoidal congestion with moderate lymphoid cellularity on 24th day (H&E, ×400)

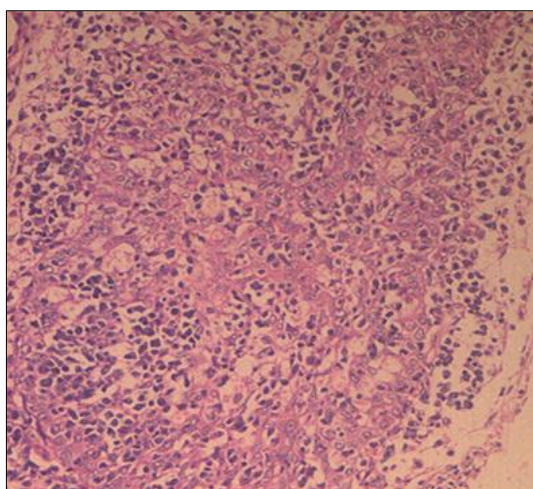


Fig 13: Bursa of Fabricius of T2 group showing moderate lymphoid depletion with interfollicular widening and reduced follicular density on 24th day (H&E, ×400)

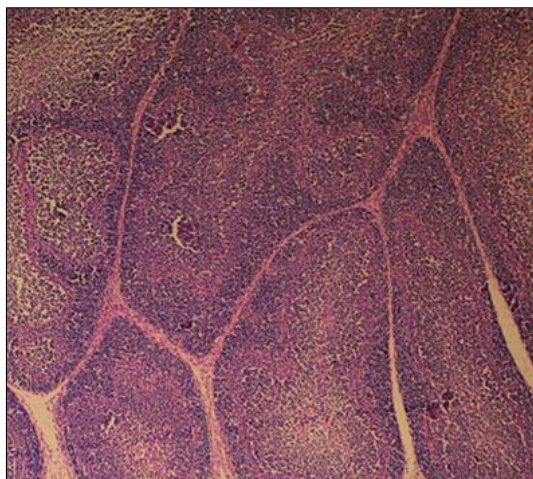


Fig 14: Bursa of Fabricius of T4 group showing moderate lymphoid cellularity with mild interfollicular widening on 24th day (H&E, ×100)

Micrometric Analysis of Jejunum and Ileum and Jejunum

Jejunal villus height differed significantly among treatment groups at both sampling intervals ($P < 0.05$) (Table 3). At

24th day, villus height was significantly lower in the infected untreated group (T_2 ; $1022.00 \pm 38.10 \mu\text{m}$) than in T_1 ($1210.33 \pm 22.50 \mu\text{m}$) and T_3 ($1154.67 \pm 50.95 \mu\text{m}$), whereas T_4 ($1083.33 \pm 25.17 \mu\text{m}$) and T_5 ($1055.00 \pm 19.05 \mu\text{m}$) recorded intermediate values. By 38th day, villus height increased in all groups; however, T_2 ($1377.00 \pm 38.11 \mu\text{m}$) remained significantly lower than T_1 ($1704.00 \pm 65.82 \mu\text{m}$), T_3 ($1739.67 \pm 77.22 \mu\text{m}$), and T_4 ($1712.00 \pm 47.03 \mu\text{m}$), which did not differ significantly from each other. T_5 recorded an intermediate villus height ($1606.67 \pm 15.28 \mu\text{m}$).

Crypt depth also differed significantly among treatment groups at both sampling intervals ($P < 0.05$). At 24th day, T_2 recorded the greatest crypt depth ($215.33 \pm 13.01 \mu\text{m}$), followed by T_5 ($207.33 \pm 9.02 \mu\text{m}$) and T_4 ($205.67 \pm 7.09 \mu\text{m}$), whereas T_1 ($182.00 \pm 4.00 \mu\text{m}$) and T_3 ($184.67 \pm 4.16 \mu\text{m}$) exhibited significantly lower values. A similar trend was observed at 38th day, with T_2 recording the greatest crypt depth ($254.67 \pm 7.02 \mu\text{m}$), followed by T_5 ($240.33 \pm 7.23 \mu\text{m}$) and T_4 ($229.33 \pm 12.06 \mu\text{m}$), while T_3 ($221.33 \pm 4.16 \mu\text{m}$) and T_1 ($212.00 \pm 5.29 \mu\text{m}$) recorded the lowest crypt depths.

The villus height-to-crypt depth (VH:CD) ratio differed significantly among treatment groups at both sampling intervals ($P < 0.05$) (Table 3). At 24th day, the lowest VH:CD ratio was observed in T_2 (4.76 ± 0.32), whereas T_1 (6.65 ± 0.23) and T_3 (6.26 ± 0.41) recorded significantly higher and comparable ratios. T_4 (5.27 ± 0.07) and T_5 (5.10 ± 0.30) exhibited intermediate values. By 38th day, T_1 (8.04 ± 0.42), T_3 (7.86 ± 0.42), and T_4 (7.48 ± 0.57) remained statistically comparable, whereas T_5 recorded an intermediate ratio (6.69 ± 0.25). T_2 continued to record the lowest VH:CD ratio (5.41 ± 0.09).

Table 3: Mean ($\pm$ SE) Jejunal villus height (μm), crypt depth (μm) and villus height to crypt depth ratio in broiler chickens at different intervals

Group	Interval					
	24 th day			38 th day		
	VH	CD	VH:CD	VH	CD	VH:CD
T_1	$1210.33^a \pm 22.50$	$182.00^b \pm 4.00$	$6.65^a \pm 0.23$	$1704.00^a \pm 65.82$	$212.00^d \pm 5.29$	$8.04^a \pm 0.42$
T_2	$1022.00^c \pm 38.10$	$215.33^a \pm 13.01$	$4.76^c \pm 0.32$	$1377.00^c \pm 38.11$	$254.67^a \pm 7.02$	$5.41^c \pm 0.09$
T_3	$1154.67^a \pm 50.95$	$184.67^b \pm 4.16$	$6.26^a \pm 0.41$	$1739.67^a \pm 77.22$	$221.33^{cd} \pm 4.16$	$7.86^a \pm 0.42$
T_4	$1083.33^b \pm 25.17$	$205.67^a \pm 7.09$	$5.27^b \pm 0.07$	$1712.00^a \pm 47.03$	$229.33^{bc} \pm 12.06$	$7.48^a \pm 0.57$
T_5	$1055.00^c \pm 19.05$	$207.33^a \pm 9.02$	$5.10^b \pm 0.30$	$1606.67^b \pm 15.28$	$240.33^b \pm 7.23$	$6.69^b \pm 0.25$
Statistics	S	S	S	S	S	S

Values are presented as mean $\pm$ SE. Means within a column bearing different superscripts differ significantly ($P < 0.05$). S – Significant

Ileum

Ileal villus height differed significantly among treatment groups at both sampling intervals ($P < 0.05$) (Table 4). At 24th day, the lowest villus height was recorded in T_2 ($668.67 \pm 11.85 \mu\text{m}$) compared with T_1 ($852.67 \pm 7.86 \mu\text{m}$), T_3 ($834.67 \pm 6.57 \mu\text{m}$), and T_4 ($829.33 \pm 10.41 \mu\text{m}$), whereas T_5 recorded an intermediate value ($723.33 \pm 5.21 \mu\text{m}$). By 38th day, T_3 ($1176.67 \pm 6.96 \mu\text{m}$) and T_4 ($1170.00 \pm 4.16 \mu\text{m}$) exhibited the greatest villus height, followed by T_1 ($1136.00 \pm 7.57 \mu\text{m}$). T_2 ($1066.00 \pm 9.87 \mu\text{m}$) remained

significantly lower than all other groups, whereas T₅ (1103.33 ± 13.48 µm) recorded intermediate values.

Crypt depth also differed significantly among treatment groups at both sampling intervals ($P < 0.05$). At 24th day, T₂ recorded the greatest crypt depth (130.33 ± 2.33 µm), followed by T₅ (119.33 ± 3.53 µm) and T₄ (111.67 ± 3.76 µm), whereas T₁ (100.00 ± 3.06 µm) and T₃ (98.33 ± 3.76 µm) recorded the lowest values. At 38th day, T₂ again recorded the greatest crypt depth (207.33 ± 3.53 µm), whereas T₄ (172.67 ± 3.53 µm) and T₅ (176.67 ± 2.91 µm) showed intermediate values. T₁ (162.67 ± 2.91 µm) and T₃

(162.33 ± 2.19 µm) remained statistically comparable and exhibited the lowest crypt depths.

The VH:CD ratio also differed significantly among treatment groups at both sampling intervals ($P < 0.05$). At 24th day, T₂ recorded the lowest ratio (5.14 ± 0.16), whereas T₁ (8.54 ± 0.30) and T₃ (8.52 ± 0.39) exhibited the highest and comparable ratios. T₄ (7.44 ± 0.16) and T₅ (6.07 ± 0.19) recorded intermediate values. By 38th day, the highest VH:CD ratio was observed in T₃ (7.25 ± 0.12), followed by T₁ (6.99 ± 0.16) and T₄ (6.78 ± 0.14). T₅ recorded an intermediate ratio (6.25 ± 0.14), whereas T₂ continued to exhibit the lowest VH:CD ratio (5.14 ± 0.08).

Table 4: Mean (± SE) Ileum villus height (µm), crypt depth (µm) and villus height to crypt depth ratio in broiler chickens at different intervals

Group	Interval					
	24 th day			38 th day		
	VH	CD	VH:CD	VH	CD	VH:CD
T ₁	852.67 ^a ± 7.86	100.00 ^c ± 3.06	8.54 ^a ± 0.30	1136.00 ^b ± 7.57	162.67 ^c ± 2.91	6.99 ^{ab} ± 0.16
T ₂	668.67 ^c ± 11.85	130.33 ^a ± 2.33	5.14 ^d ± 0.16	1066.00 ^d ± 9.87	207.33 ^a ± 3.53	5.14 ^d ± 0.08
T ₃	834.67 ^a ± 6.57	98.33 ^c ± 3.76	8.52 ^a ± 0.39	1176.67 ^a ± 6.96	162.33 ^c ± 2.19	7.25 ^a ± 0.12
T ₄	829.33 ^a ± 10.41	111.67 ^b ± 3.76	7.44 ^b ± 0.16	1170.00 ^a ± 4.16	172.67 ^b ± 3.53	6.78 ^b ± 0.14
T ₅	723.33 ^b ± 5.21	119.33 ^b ± 3.53	6.07 ^c ± 0.19	1103.33 ^c ± 13.48	176.67 ^b ± 2.91	6.25 ^c ± 0.14
Statistics	S	S	S	S	S	S

Values are presented as mean ± SE. Means within a column bearing different superscripts differ significantly ($P < 0.05$). S – Significant

Re-isolation and Enumeration of *Clostridium perfringens*

Experimental *Clostridium perfringens* was successfully re-isolated from the ileo-caecal contents of challenged birds on the 24th and 38th day. Typical black sulphite-reducing colonies were observed on Tryptose Sulphite Cycloserine (TSC) agar, while the isolates produced characteristic opalescent zones on egg yolk agar and smooth grey colonies with a double zone of haemolysis on blood agar. Gram staining revealed large Gram-positive rods arranged singly or in short chains, and all isolates were catalase-negative, confirming their identity as *C. perfringens* (Figure X).

Intestinal *C. perfringens* counts are presented in Table 5. Significant differences in bacterial counts were observed among treatment groups at both sampling intervals ($P < 0.05$). On the 24th day, T₂ recorded the highest bacterial load (6.58 ± 0.49 log₁₀ CFU/g), followed by T₅ (6.13 ± 0.14 log₁₀ CFU/g). Lower counts were observed in T₄ (5.15 ± 0.09 log₁₀ CFU/g) and T₃ (5.08 ± 0.11 log₁₀ CFU/g), whereas T₁ recorded the lowest count (3.60 ± 0.08 log₁₀ CFU/g). By the 38th day, bacterial counts had decreased in all treatment groups; however, T₂ continued to record the highest count (6.15 ± 0.17 log₁₀ CFU/g). Among the treated groups, T₅ recorded higher counts (5.19 ± 0.12 log₁₀ CFU/g) than T₄ (4.14 ± 0.08 log₁₀ CFU/g) and T₃ (4.07 ± 0.11 log₁₀ CFU/g), which did not differ significantly from each other. The lowest bacterial count on the 38th day was recorded in T₁ (3.68 ± 0.08 log₁₀ CFU/g).

Table 5: Mean (± SE) log₁₀ CFU/g of intestinal *Clostridium perfringens* in broiler chickens at different intervals

Group	24 th Day	38 th Day
T ₁	3.60 ^c ± 0.08	3.68 ^d ± 0.08
T ₂	6.58 ^a ± 0.49	6.15 ^a ± 0.17
T ₃	5.08 ^b ± 0.11	4.07 ^c ± 0.11
T ₄	5.15 ^b ± 0.09	4.14 ^c ± 0.08
T ₅	6.13 ^a ± 0.14	5.19 ^b ± 0.12

Values are presented as mean ± SE. Means within a column bearing different superscripts differ significantly ($P < 0.05$). CFU = colony-forming units.

Discussion

The present study successfully established an experimental necrotic enteritis (NE) model following oral challenge with *Clostridium perfringens*, as demonstrated by characteristic clinical signs, gross and histopathological lesions, increased intestinal bacterial counts, altered intestinal morphology, impaired growth performance, and changes in serum biochemical parameters. Among the treatment groups, prophylactic supplementation with *Saccharomyces boulardii* consistently produced greater improvement than therapeutic supplementation and showed efficacy comparable to enramycin for most evaluated parameters.

Experimental *C. perfringens* infection markedly impaired growth performance, as evidenced by reduced feed intake, body weight, and body weight gain together with poorer feed conversion ratio. These changes were accompanied by significantly higher intestinal *C. perfringens* counts, indicating successful bacterial colonization. Similar reductions in productive performance following experimental NE have been reported by Jayaraman *et al.* (2013) [26], Shojadoost *et al.* (2012) [3], and Wu *et al.* (2018) [27]. Birds receiving enramycin and prophylactic *S. boulardii* exhibited lower intestinal bacterial counts together with improved productive performance, whereas therapeutic supplementation produced comparatively moderate improvement. Similar preventive effects of probiotic supplementation have been reported by Nari (2020) [28] and Caly *et al.* (2015) [21].

Gross and histopathological examination of the infected untreated group revealed classical lesions of NE, including intestinal distension, mucosal congestion, hemorrhages, fibrinonecrotic pseudomembrane formation, villus necrosis, epithelial desquamation, inflammatory cell infiltration, and lesions in the liver, kidneys, spleen, and bursa of Fabricius. These observations are consistent with previous descriptions of experimental NE by Shojadoost *et al.* (2012) [3], Keyburn *et al.* (2008) [7], and Mwangi *et al.* (2019) [29]. The pathological findings were supported by intestinal morphometry, where infected untreated birds exhibited

reduced villus height, increased crypt depth, and a lower villus height-to-crypt depth (VH:CD) ratio in both the jejunum and ileum. In contrast, enramycin and prophylactic *S. boulardii* preserved intestinal architecture, with morphometric measurements approaching those of the negative control. Similar improvements in intestinal morphology following probiotic supplementation have been reported by Jayaraman *et al.* (2013) [26], Wu *et al.* (2018) [27], and Nari (2020) [28]. The comparatively lower VH:CD ratio in the therapeutic group further indicates that supplementation after disease establishment was less effective than preventive administration.

Serum biochemical alterations further reflected the systemic effects of NE. The infected untreated birds exhibited reduced serum total protein, globulin, and cholesterol concentrations together with increased ALT, AST, and creatinine activities, whereas enramycin- and prophylactic *S. boulardii*-treated birds maintained biochemical values closer to those of the negative control. Comparable biochemical alterations during experimental *C. perfringens* infection have been reported by Song *et al.* (2017) [30], Jayaraman *et al.* (2013) [26], and Wu *et al.* (2018) [27]. The comparatively higher serum protein and globulin concentrations together with lower serum transaminase activities observed in the treated groups corresponded with the reduced intestinal and hepatic lesions observed in the present study.

Overall, the findings demonstrate that prophylactic supplementation with *S. boulardii* effectively reduced intestinal colonization by *C. perfringens*, preserved intestinal architecture, improved productive performance, and minimized biochemical alterations associated with experimental necrotic enteritis. Its protective efficacy was comparable to enramycin for most evaluated parameters, supporting its potential as a non-antibiotic strategy for the prevention of necrotic enteritis in broiler chickens.

Conclusion

The present study demonstrated that experimental challenge with *Clostridium perfringens* successfully induced necrotic enteritis in broiler chickens, resulting in impaired growth performance, alterations in serum biochemical parameters, severe gross and histopathological intestinal lesions, disruption of intestinal morphometry, and increased intestinal bacterial colonization. Dietary supplementation with *Saccharomyces boulardii* effectively mitigated these adverse effects, with prophylactic administration consistently producing greater protective effects than therapeutic supplementation. Birds receiving prophylactic *S. boulardii* exhibited improved growth performance, preservation of intestinal architecture, lower intestinal *C. perfringens* counts, reduced lesion severity, and maintenance of serum biochemical parameters comparable to those observed in the enramycin-treated group. Overall, the findings demonstrate that prophylactic *S. boulardii* supplementation is an effective non-antibiotic strategy for reducing the severity of necrotic enteritis and maintaining intestinal health and productivity in broiler chickens. These results support its potential application as a sustainable alternative to antibiotic growth promoters in commercial poultry production.

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