


EFFECTS OF DIETARY YEAST CELL WALL SUPPLEMENTATION ON BROILER CHICKEN PERFORMANCE AND GUT HEALTH

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ABSTRACT

This study investigated the effects of dietary yeast supplementation on anticoccidial activity, antioxidant status, performance, and blood parameters in broiler chickens challenged with coccidiosis. A total of 375 one-day-old broiler chicks were randomly assigned to five experimental groups, with three replicates per group (25 chicks per replicate). Treatments included two yeast-supplemented groups (Ty1: 1 g/kg; Ty2: 3 g/kg), two negative control groups (unchallenged, untreated and challenged, untreated), and a positive control group treated with sulphonamide and diaveridine HCl. Birds were raised under standard management conditions for 35 days. Performance parameters, including body weight gain, feed intake, and feed conversion ratio, were recorded. Anticoccidial efficacy was assessed via lesion scoring and oocyst index, while additional evaluations included meat quality traits, blood biochemical profiles, and immune organ development. Yeast supplementation, particularly at 3 g/kg (Ty2), significantly reduced oocyst shedding ($P < 0.01$) and mitigated intestinal lesions compared with infected controls. This was supported by histopathological findings in the Ty2 group, which showed preserved intestinal architecture with reduced necrosis. Enhanced meat quality was also observed, including improvements in water-holding capacity, cooking loss, and fillet weight. No adverse effects were noted in blood biochemistry or immune organ weights, indicating systemic safety. It was concluded that dietary yeast, especially at 3 g/kg, effectively improved gut health and meat quality, supporting its potential as a natural alternative for coccidiosis control in broiler production.

Keywords: Yeast supplementation, Coccidiosis, Broiler chickens, Anticoccidial activity, Meat quality, Immune response.

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1. INTRODUCTION

Coccidiosis remains one of the most economically devastating parasitic diseases affecting global poultry production, caused by protozoan parasites of the genus *Eimeria*. The disease impairs nutrient absorption due to intestinal damage, resulting in reduced growth performance, poor feed conversion, increased morbidity, and mortality (Shivaramaiah et al., 2021). Control strategies have traditionally relied on anticoccidial drugs such as sulphonamides and ionophores. However, growing concerns regarding antimicrobial resistance, drug residues in poultry products, and increasingly stringent regulatory restrictions have intensified the search for natural, sustainable alternatives (Wu et al., 2021; El-Shall et al., 2022; M'Sadeq et al., 2023). Coccidiosis causes intestinal leakage of plasma protein, malabsorption, villous atrophy, interruption of nutrient digestion, decreased weight gain (WG), reduced feed and water intake, increased feed conversion ratio (FCR), increased mortality rates, increased intestinal passage, increased susceptibility to other diseases, and increased medication expenses (Martins et al., 2022).

This search for alternatives has focused on natural agents with immunomodulatory and antimicrobial properties, among which botanical compounds have emerged as a particularly promising frontier. Aromatic plants and their extracts are rich in bioactive compounds with significant therapeutic potential against parasitic diseases (Smith, 2023). This is evidenced by recent studies demonstrating the efficacy of specific botanicals against coccidial infection in poultry. For example, the ethanolic extract of clove (*Syzygium aromaticum*) and the essential oil of *Amomum subulatum* have shown strong anticoccidial activities in broiler chickens, improving health outcomes and reducing parasitic load (Zaheer et al., 2022; Khan et al., 2023a). Similarly, *Linum usitatissimum* (flaxseed) essential oil exhibits growth-promoting, hematological, and anticoccidial potential. The principle of botanical control is effective across species, as shown in ruminants (Iqbal et al., 2023), and the biochemical potential of plants, even under stress, underscores their robustness as a source of bioactive compounds (Ali et al., 2023). The mechanism of action of many botanicals, including the nematocidal effects of *Khaya senegalensis* and *Azadirachta indica* against *Haemonchus contortus* (Idris et al., 2023), often involves disrupting parasite integrity

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58 and modulating host immunity, providing a solid rationale for exploring other natural sources, such as yeast.

59 Yeast and yeast-derived products have garnered considerable interest as multifunctional feed additives in
60 poultry nutrition. Yeast components such as β -glucans, mannan-oligosaccharides (MOS), nucleotides, and enzymes
61 exhibit immunomodulatory, antimicrobial, and antioxidant properties (Alizadeh et al., 2021; Song et al., 2023).
62 These bioactive compounds enhance gut health by promoting beneficial microbiota, improving intestinal integrity,
63 and stimulating both innate and adaptive immune responses (Perricone et al., 2022). As a result, dietary yeast has
64 shown potential to mitigate enteric diseases such as coccidiosis while also enhancing nutrient absorption and
65 performance. Various feed additives—including yeast, probiotics, prebiotics, organic acids, and plant extracts—
66 have been explored to enhance poultry productivity, immunity, and control coccidiosis (Peek & Landman, 2011; Wu
67 et al., 2021; Khan et al., 2023b). *Saccharomyces cerevisiae* yeast cell walls (YCW), rich in β -glucans and mannan-
68 oligosaccharides (MOS), provide immunomodulatory effects and improve gut health by limiting pathogen colonization
69 (Shanmugasundaram et al., 2013a; 2013b; Alizadeh et al., 2021; Magnoli et al., 2024). With strict regulations on
70 chemical anticoccidials in Europe since 2006, natural alternatives like YCW and plant-derived additives are gaining
71 increased use and attention (Hayajneh et al., 2018a; 2018b; El-Shall et al., 2022).

72 Recent studies have reported that yeast supplementation improves weight gain, reduces oocyst shedding, and
73 enhances villus morphology in broilers challenged with *Eimeria* spp. (Chen et al., 2022; Amer et al., 2023).
74 Additionally, improvements in carcass traits, meat water-holding capacity, and oxidative stability have been
75 associated with yeast-derived polysaccharides and antioxidants (Kiczorowska et al., 2020; Hosseini Mansoub et
76 al., 2021). While previous studies have demonstrated the general benefits of yeast supplementation, the novel
77 aspect of this study is the direct, comparative evaluation of two distinct yeast cell wall dosages (1g/kg vs. 3 g/kg)
78 to determine the optimal level for mitigating specific coccidiosis-related pathologies under an experimental
79 challenge model. This precise dosage optimization remains unexplored and is crucial for practical application in
80 the feed industry.

81 Therefore, we hypothesized that dietary supplementation with YCW would confer dose-dependent protective
82 effects against coccidiosis, with the higher dose (3 g/kg) demonstrating superior efficacy in enhancing growth
83 performance, reducing oocyst shedding and lesion scores, improving antioxidant status, and modulating immune
84 responses compared to the lower dose and the infected, un-supplemented control.

85 This study is significant because it moves beyond establishing mere efficacy and provides actionable data on
86 optimal dosage. The findings will offer valuable insights for developing effective, natural strategies to control
87 coccidiosis, thereby reducing reliance on anticoccidial drugs and contributing to more sustainable and safe poultry
88 production systems.

90 2. MATERIALS AND METHODS

91 2.1. Study Place

92 This research was conducted at the poultry production unit of the Jordan University Station for Dry Land
93 Research in Al-Muwaqqar, located 45 km southeast of Amman, Jordan. The closed house consisted of 10 rooms.
94 Each room is further divided into 3 chambers, each equipped with stoves, drinkers, feed troughs, and a lighting
95 system.

96 2.2. Management of Experimental Birds

97 All chicks had a close initial body weight (48 ± 0.05 g). Feed and water were provided ad libitum. Chicks were
98 reared on a floor covered with wood shavings. Nutrient requirements and management practices were applied in
99 accordance with breed guidelines. Chicks were reared according to standard management practices. Light was
100 provided for 24 h throughout the experimental period (as recommended by the Ross 308 rearing guide). All chicks
101 were fed a broiler starter ration until week 2, then a finisher ration (Table 1). The pen temperature was kept at 29-
102 32°C during the first week of age; thereafter, it was reduced by 0.5°C weekly. The birds were assigned to their
103 groups on day 12, and the medications were added to the feed on that day. The birds were vaccinated for infectious
104 bronchitis on day 1 and for Newcastle disease on day 7 (Hayajneh et al., 2024).

105 2.3. Experimental Design

106 Chicks were divided into 5 groups, each group composed of three replicates, and each replicate had 25 chicks.
107 Treatments were arranged in a Randomized Complete Block Design (RCBD) with three replicates.

110

111 2.4. Drugs and Administration

112 On day 12 the drugs were given to birds daily: sulfadimidine (320 mg) and diaverdine HCl (10 mg) (Avico,
113 Amman, Jordan) was administered to birds in the T_{+ve} group at a concentration of 100 mg/200L drinking water for
114 three days, followed by plain water /2 days, then the medication was added in water at 100 mg/200L water/2 days).
115 Yeast was given to group T_{y1} at a concentration of 1 g/kg feed/7 days, and to group T_{y2} at 3 g/kg/7 days. Nothing

116 was added to T_{-ve1} and T_{-ve2} (Table 2). Two yeast concentrations were added to diets at the feed mill.
117

Table 1: Composition of broiler basal feed (g/kg) 118

Ingredients	Starter (0-21 d)	Finisher (22-35 d)	119
Maize	540.0	710.0	120
Soybean meal (480 g/kg CP)	360	250.0	121
Dicalcium phosphate	20	20.0	122
Soybean oil	13	13.0	123
Fish meal	60	0	124
DL-Methionine	1.5	0.5	125
Vitamin-mineral premix ^{a,b}	3.5	3.5	126
Sodium chloride	2.0	3.0	127
Chemical composition (g/kg diet as fed basis)			128
MEn (MJ/kg)	12.6	13.0	129
Crude protein	235.0	182.0	130
Calcium	12.3	8.0	131
Total Phosphorus	6.5	5.1	132
Lysine	17.0	10.0	133
Methionine	9.0	8.0	134

^aVitamin premix provided per kilogram of diet: Vitamin A = 0.5760; Vitamin K = ?IU; Vitamin B5 = 19.4532mg; Tocopherol = 4.6799mg; Vitamin B1 = 3.5016mg; Vitamin B2 = 1.6994mg; Vitamin B6 = 6.4911mg; Vitamin B12 = 16mg; Biotin = 0.1382mg; Folic Acid = 1.2692mg; Pantothenic = 7.8104mg; Vitamin K3 = 0.9071mg; ^bTrace mineral premix per kg diet were: Iron = 62.0061mg; Zinc = 43.065mg; Copper = 6.8539mg; Iodine = 0.0589mg; Selenium = 1.3466mg. Soybean concentrate and mono-calcium phosphate were also provided. 140

141 horizontal mixer for at least 15 minutes to ensure homogeneous distribution of the supplement. The control diets were prepared using the same procedure, without the addition of yeast.

Table 2: Group arrangement in the experiment

Group	Drug Used	Dose	Coccidiosis Introduced
T _{y1}	Infected medicated with yeast at 1 g/kg feed	1 g/kg	Yes
T _{y2}	Infected medicated with yeast at 3 g/kg feed	3 g/kg	Yes
T _{+ve}	sulphadimidine 320 mg and diaverdine HCL 10 mg	100 mg/200l drinking water for three days, followed by plain water/2days, then the medication was added in water 100 mg/200l water/2days	Yes
T _{-ve1}	Infected medicated with bentonite 1.5 mg/kg	1.5 mg/kg	No
T _{-ve2}	None infected; none medicated	None	No

146 This study was designed to evaluate the prophylactic efficacy of yeast cell wall supplementation against coccidiosis. All medications (yeast and the conventional drug) were initiated on day 12, while coccidiosis challenge was administered on day 14. This two-day pretreatment period reflects commercial practice, in which feed additives are continuously included before disease exposure to prevent outbreaks. Anticoccidial drugs are also commonly used prophylactically in poultry production; therefore, the positive control comparison is valid and appropriate, as all groups received interventions under the same preventive protocol. 152

Table 3: Analyzed composition of the yeast cell wall supplement (as-fed basis)

Component	Value (%)
Mannan Oligosaccharides (MOS)	22.5
β-Glucans	18.0
Crude Protein	16.0
Crude Fat	5.5
Ash	8.0
Moisture	6.0

167 **2.6. Evaluation of Sensitivity or Resistance**

168 Seventeen chicks were randomly selected from each group on day 12, weighed, and then reweighed on day 21;

2.5. Yeast Supplement: Source, Composition, and Preparation

The yeast-based supplement used in this study was a commercial dried yeast cell wall product derived from the fermentation of *Saccharomyces cerevisiae*. The product was sourced from Jordan Bio-Industries Center (JOBIC), Amman, Jordan. The product appeared as a fine, tan-to-beige colored powder. Its typical nutritional composition as provided by the manufacturer (Table 3).

The yeast-based supplement used in this study was a commercial, dried yeast cell wall product derived from the fermentation of *Saccharomyces cerevisiae*. The product was sourced from Jordan Bio-Industries Center (JOBIC) in Amman, Jordan, a representative supplier of yeast-based feed additives in the region. The product was a fine, tan-to-beige-colored powder. The product was stored in a cool, dry place, protected from direct sunlight until use. For inclusion in the experimental diets, the required amount of yeast powder was first carefully mixed with a small portion of the basal corn-soybean meal diet to form a uniform premix. This premix was then gradually incorporated into the complete feed and thoroughly blended using a

141 horizontal mixer for at least 15 minutes to ensure homogeneous distribution of the supplement. The control diets were prepared using the same procedure, without the addition of yeast.

157 **2.5. Histopathological Examination**

158 Tissue samples from morbid intestines were collected for histopathological studies. Tissue samples were preserved in 10% buffered formalin and processed for histopathological studies using routine methods of dehydration and paraffin embedding. Sections 4–5µm thick were cut and stained with hematoxylin and eosin (Hayajneh et al., 2020). Slides were examined under a light microscope. 166

169 the BWT gain difference was calculated between these days. Five chicks from each group were sacrificed on day 21,
170 and the intestinal lesion score was recorded (Raman et al., 2011). The gross lesion scores (GLS) were assessed on a
171 scale of 0 (no gross lesion) to 4 (most severe lesion). After microscopic examination of intestinal scrapings taken from
172 sacrificed birds, the oocyte index was determined (0-5) as described by Arabkhazaeli et al. (2013). The sensitivity of
173 drugs was determined using the global index (GI), and the global index (GI) was calculated (Abbas et al., 2009).
174

175 2.7. Coccidiosis Induction

176 Birds infected with coccidiosis were naturally collected from local farms, and infectious coccidial oocysts were
177 isolated, as described by Hayajneh et al. (2020), according to the site of infection and oocyst morphology, including
178 size, shape, and color after sporulation, five species were identified: *E. acervulina* (10%), *E. brunette* (13%), *E.*
179 *maxima* (12%), *E. necatrix* (12%), and *E. tenella* (57%). On day 14, coccidiosis was introduced in broiler chicks via
180 the oral route, and each chicken received an infectious coccidian dose of 3×10^5 /bird (Abbas et al., 2011).
181

182 2.8. Performance Parameters

183 Feed intake, body weight gain, and feed conversion ratio (FCR) were calculated on days 21, 28, and 35. Also,
184 oocysts per gram of feces, lesion score, and oocyst scoring were recorded on days 14, 21, and 28. Health aspects
185 and mortality were monitored daily. Efficacy status was calculated as a percentage of the NNC's GI. Meat quality
186 was determined according to Jalal et al. (2023).
187

188 2.9. Lesion scoring and Oocyte Index

189 On days 7, 14, and 21 post-inoculation (experimental days 21, 28, and 35), three birds from each replicate were
190 randomly selected and killed humanely for coccidial lesion scoring. A score of 0 to +4 was recorded for each chicken
191 (Abas et al., 2009; Raman et al., 2011). An Oocyst index of 0 to 5 was determined after examining scrapings from
192 birds sacrificed for lesion scoring. Oocyte index was determined (Abbas et al., 2009; Arabkhazaeli et al., 2013).
193

194 2.10. Measurements

195 Magnesium, creatinine, and uric acid were measured using ELISA kits from Biolab, Jordan. Albumin,
196 cholesterol, magnesium, total protein, bilirubin, and phosphorus were measured using an ELISA kit from Biomed,
197 MDSS GmbH, Germany. Glucose was measured using an ELISA kit from Atlas Medical Jordan. Uric acid using an
198 ELISA kit from ARCOMEX Jordan. Zinc was measured using a spectrophotometry kit from Ita, Italy.
199 Malondialdehyde and total antioxidant capacity were measured using assay kits from Creative Proteomics, USA. To
200 measure Newcastle disease and infectious bursal disease antibodies in serum, a commercial ELISA kit from
201 BioChek Immunoassays (BioCheck, Reeuwijk, Netherlands) was used according to the manufacturer's guidelines.
202 The cut-off used was <0.35 . The tests were performed at Feedco Labs in Jordan. Villus surface area was calculated
203 using the formula = $(2) \times (VW/2) \times (VH)$, in which VW = villus width and VH = villus height (De los et al., 2005).
204 A total of 15 villus areas were calculated for each bird.
205

206 2.11. Statistical Analysis

207 SPSS software was used to analyze the collected data. One-way analysis of variance was used, and the means
208 of the different treatments were compared using Tukey's test and a repeated-measures test. Values were considered
209 statistically different at $P \leq 0.05$, and the mean values were compared using Tukey's test.
210

211 3. RESULTS

212 No significant differences were observed in the weights of the liver and bursa among groups ($P > 0.05$), whereas
213 spleen weights differed significantly ($P = 0.040$), potentially reflecting immune-modulatory effects of the yeast
214 treatment (Table 4). Additionally, blood biochemical parameters, including albumin, glucose, cholesterol, total
215 protein, uric acid, magnesium, phosphorus, and zinc, showed no statistically significant differences between groups
216 ($P > 0.05$; Table 5), suggesting that yeast inclusion at both doses did not adversely affect systemic physiology.
217

218 **Table 4:** Mean weight of liver, spleen and bursa in the different groups

	Sum of Squares	Mean Square	F	P value
Liver	194.889	24.361	0.342	0.948
Spleen	3.824	0.478	2.092	0.040
Bursa	69.142	8.643	1.062	0.392

Based on observed means (df = 8). A significant level was considered at $P < 0.05$.

221 Overall, dietary yeast supplementation, particularly at 3g/feed (Ty2), improved meat quality and reduced
222 coccidial lesions without negatively impacting growth performance or health indicators.

223 Broiler chickens in the yeast-treated groups (Ty1: 1 g/kg; Ty2: 3 g/kg) exhibited distinct responses across multiple

224 parameters. Lesion scoring and oocyst index were significantly reduced in both yeast-treated groups compared to
 225 the infected untreated controls, indicating marked anticoccidial activity ($P < 0.01$; Table 6). Although weight gain
 226 and feed intake did not differ significantly among groups ($P < 0.05$), Ty2 showed a numerically higher average
 227 weight gain compared to Ty1 (Table 6).

228 Regarding meat quality traits, both Ty1 and Ty2 exhibited statistically significant differences in meat weight
 229 (MWT), fillet weight (FWT), lightness (L), yellowness (B), cooking loss (CL), water holding capacity (WHC), and
 230 shear force (SHF) compared to other groups ($P < 0.001$; Table 7). Notably, Ty2 yielded higher meat and fillet
 231 weights, whereas Ty1 achieved the highest WHC values, suggesting enhanced juiciness. Redness (a^*) and pH
 232 values did not differ significantly ($P > 0.05$), indicating that yeast supplementation did not affect meat color stability
 233 or acid-base status.

234
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Table 5: Blood parameters in the different groups

	Sum of Squares	Mean Square	F	P value.
Albumin	0.115	0.014	1.306	0.255
Cholesterol	2318.673	289.834	0.857	0.557
Glucose	15030.612	1878.827	1.116	0.363
Total Protein	0.595	0.074	0.198	0.990
Magnesium	17.644	2.205	1.196	0.314
Uric Acid	4.718	0.590	0.344	0.946
Phosphorus	150.839	18.855	1.099	0.374
zinc	24484.425	3060.553	1.693	0.116
IB Titer	1339151.556	167393.944	1.202	0.310
ND concentration	512002.691	64000.336	0.565	0.803

236 For ND (Newcastle disease virus titer) and IB (infectious bursal disease virus titer), results were based on observed means
 237 ($df = 8$). The error term was $MSE = 113254.756$. *. A significant level was considered at $P < 0.05$. For the remaining blood
 238 parameters, virus results were based on observed means. The error term is Mean Square (Error) = 1808.239. *.

239
240

Table 6: Mean values of lesion scoring and performance parameters in the different groups

	Sum of Squares	Mean Square	F	P value
Lesion score	22.519	2.815	5.846	0.001
Oocyte index	70.963	8.870	19.958	0.000
Mortality	15.630	1.954	0.676	0.706
Weight gain	127826.775	15978.347	0.803	0.608
Feed intake	149809.895	18726.237	0.829	0.588

241 Based on observed means ($df = 8$). The error term is Mean Square (Error) = 22578.388. A significant level was considered at
 242 $P < 0.05$.

243
244

Table 7: Meat quality values in the different groups

	Sum of Squares	Mean Square	F	P value
MWT	56671.760	7083.970	19.223	0.000
FWT	49835.920	6229.490	23.645	0.000
L	2234686.667	279335.833	12.569	0.000
A	5343475.728	667934.466	1.316	0.250
B	818197.580	102274.698	4.041	0.001
PH	.003	0.000	.737	0.658
CL	202.552	25.319	12.107	0.000
WHC	399.512	49.939	34.140	0.000
SHF	15.970	1.996	5.766	0.000

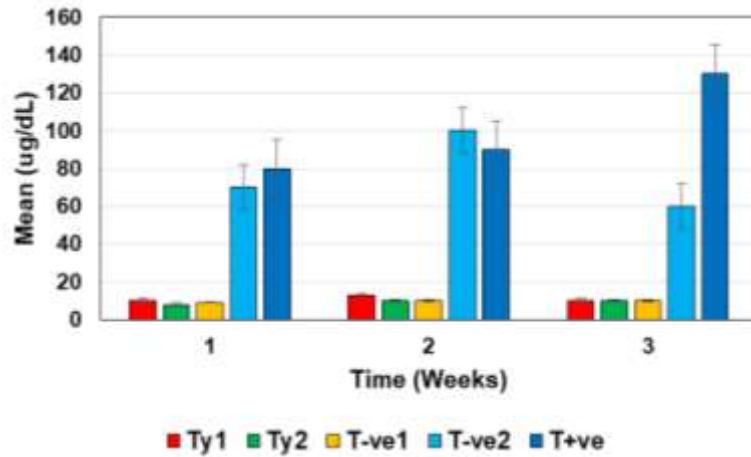
245 Based on observed means ($df = 8$). The error term is Mean Square (Error) = 0.346. A significant level was considered at
 246 $P < 0.05$. MWT: Muscle weight; Fillet weight: weight of the breast fillet; L, A, B: meat color parameters in the CIE Lab system (L
 247 = lightness, A = red/green, B = yellow/blue); pH: muscle acidity measured postmortem; CL: cooking loss (%); Water holding
 248 capacity (WHC): ability of meat to retain water; Shear force: indicator of meat tenderness.

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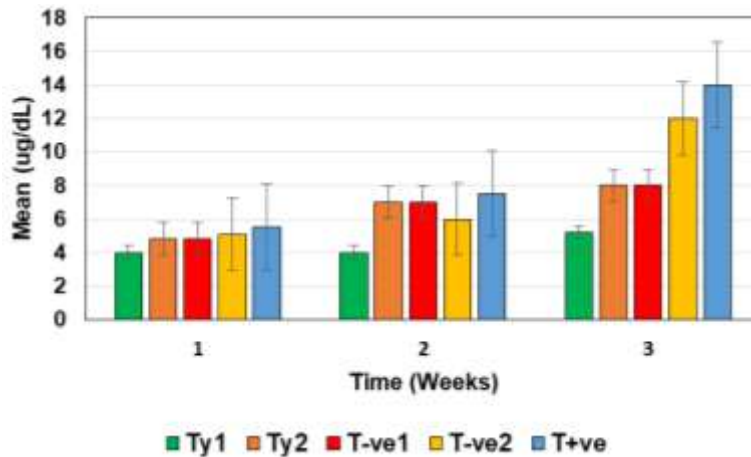
Histopathological examination of intestinal sections supported the findings of the gross lesion and oocyst
 251 index. Severe epithelial damage, extensive schizont stages, and marked necrosis were observed in the infected
 252 untreated groups (T-ve1 and T-ve2), indicating heavy coccidial infection. In contrast, birds supplemented with yeast
 253 at 1 g/kg (Ty1) showed moderate pathological changes, with partial villus damage, while those receiving 3 g/kg of
 254 yeast (Ty2) exhibited relatively preserved intestinal architecture, with reduced necrosis and fewer parasitic stages.

255 Total antioxidant capacity differed among the experimental groups, as illustrated in Fig. 1. Yeast
 256 supplementation significantly enhanced antioxidant status in broiler chickens challenged with coccidiosis, with the
 257 highest total antioxidant capacity observed in the Ty2 group (3 g/kg yeast). Lipid peroxidation, as indicated by

258 malondialdehyde levels are presented in Fig. 2. Yeast supplementation reduced malondialdehyde concentrations
 259 compared with the infected untreated control, with a more pronounced reduction in birds receiving 3g/yeast,
 260 indicating attenuation of oxidative stress.
 261



262
 263 **Fig. 1:** Total antioxidant capacity (µg/dL) in different experimental groups. Data are based on n = 8. Bars bearing bar errors
 264 falling out with others, differing significantly (P<0.05) over time. Ty1 = Infected medicated with yeast at 1 g/kg feed; Ty2 =
 265 Infected medicated with yeast at 3 g/kg feed; T+ve = Sulphadimidine 320mg and diaverdine HCL 10mg; T-ve1 = Infected
 266 medicated with bentonite 1.5m g/kg; T-ve2 = None infected, none medicated.
 267



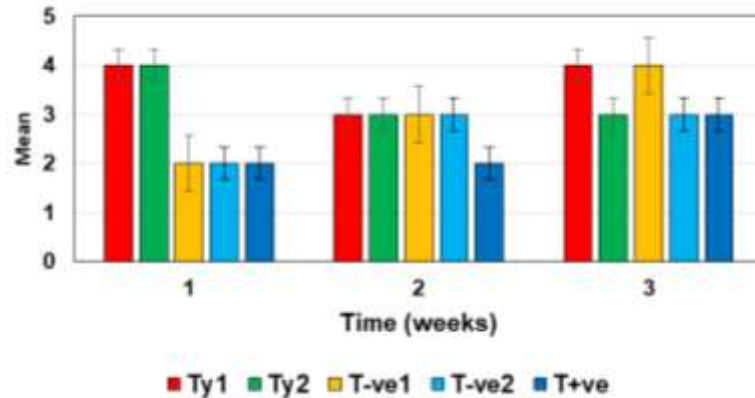
268
 269 **Fig. 2:** Malondialdehyde concentration (µg/dL) capacity in different experimental groups. Data are based on n = 8. Bars bearing
 270 bar errors falling out with others, differing significantly (P<0.05) over time. Ty1 = Infected medicated with yeast at 1 g/kg feed;
 271 Ty2 = Infected medicated with yeast at 3 g/kg feed; T+ve = Sulphadimidine 320mg and diaverdine HCL 10mg; T-ve1 = Infected
 272 medicated with bentonite 1.5m g/kg; T-ve2 = None infected, none medicated.
 273
 274

275 4. DISCUSSION

276 Supplementation of broiler diets with two distinct yeast doses (Ty1 at 1 g/kg and Ty2 at 3 g/kg) significantly
 277 improved meat quality and reduced coccidial lesions without impairing growth performance or systemic health.
 278 More importantly, this direct comparative analysis provides crucial, novel insights into the dose-dependent
 279 efficacy of yeast supplementation, addressing a key gap in existing literature. These findings align with and
 280 extend previous studies highlighting the multifaceted benefits of yeast-derived additives in poultry production
 281 (Bortoluzzi et al., 2019; Liu et al., 2023).

282 Our findings on the efficacy of yeast cell walls (YCW) in reducing oocyst shedding and lesion scores (Fig. 3)
 283 are consistent with the growing evidence supporting natural alternatives to chemical anticoccidials. The reduction in
 284 pathogenicity observed in our study parallels the effects reported for several plant-derived bioactives. For instance,
 285 ethanolic extracts of clove (*Syzygium aromaticum*) and essential oil of *Amomum subulatum* have demonstrated
 286 significant anticoccidial activity in broiler chickens, likely through antimicrobial and anti-inflammatory
 287 mechanisms (Khan et al., 2023a; Zaheer et al., 2022). Similarly, *Linum usitatissimum* (flaxseed) essential oil has

288 been shown to improve hematological parameters and growth performance, suggesting systemic health benefits
 289 comparable to the immunostimulatory effects indicated by increased spleen weight in our YCW-supplemented
 290 groups. Although the active components differ— β -glucans and mannan oligosaccharides (MOS) in yeast versus
 291 phenolic compounds and essential oils in plants—both approaches share the same objective: strengthening the
 292 host's innate immunity and creating a hostile environment for the parasite, without the adverse effects associated
 293 with conventional drugs. Collectively, these findings highlight those natural strategies, whether fungal or botanical,
 294 offer a promising and sustainable alternative for coccidiosis control.
 295
 296



297
 298 **Fig. 3:** Coccidia lesion score in different groups. Data are based on n = 8. Bars bearing bar errors falling out with others,
 299 differing significantly ($P < 0.05$) over time; Ty1 = Infected medicated with yeast at 1 g/kg feed; Ty2 = Infected medicated with
 300 yeast at 3 g/kg feed; T+ve = Sulphadimidine 320mg and diaverdine HCL 10mg; T-ve1 = Infected medicated with bentonite 1.5m
 301 g/kg; T-ve2 = None infected, none medicated.
 302

303 The significant reduction in lesion scores and oocyst shedding observed in both yeast-treated groups supports
 304 the established role of yeast cell wall components as effective anticoccidial agents. Giannenas et al. (2023) reported
 305 that multi-strain yeast fractions improved intestinal health under challenge. Findings of the present study confirm
 306 and further demonstrate that a higher dose (Ty2) elicited a more robust reduction in pathological markers,
 307 underscoring the importance of dosage optimization for maximum anticoccidial effect.

308 Regarding meat quality, both Ty1 and Ty2 significantly improved meat and fillet weights, WHC, and
 309 tenderness. A key novel finding was the differential impact on meat traits: while Ty2 improved overall yield, Ty1
 310 showed superior water-holding capacity. This suggests that moderate yeast supplementation may enhance meat
 311 juiciness more effectively, whereas higher doses favor mass, a dose-response relationship that refines the
 312 application guidelines for producers. These improvements are consistent with findings from Bai et al. (2022) and
 313 Kim et al. (2022), but our study is the first to directly contrast these outcomes at different inclusion levels under a
 314 standardized coccidial challenge.

315 Although growth performance parameters were not statistically different, the trend toward improved average
 316 daily gain in Ty2 aligns with previous findings where yeast enhanced nutrient digestibility under stress (Abd El-
 317 Hack et al., 2022). Maintaining growth performance, even during a disease challenge, is a significant result,
 318 confirming that yeast supplementation does not impose a metabolic cost and protects productivity.

319 The lack of significant variation in blood biochemical parameters and organ weights confirms the systemic
 320 safety of yeast supplementation. The significant increase in spleen weight in yeast-fed groups likely reflects a
 321 beneficial immunostimulatory effect, as reported by Song et al. (2021). This finding holds practical significance, as
 322 it indicates an enhanced immune readiness without the pathology of inflammation, a valuable trait in disease-prone
 323 commercial settings.

324 Overall, the core novelty of this study—the dose-comparison—reveals that while both doses are beneficial, Ty2
 325 (3 g/kg) provided the most comprehensive anticoccidial protection and improved meat yield, whereas Ty1 (1 g/kg)
 326 offered specific advantages for meat quality traits like water retention. This provides the industry with actionable
 327 data to select a dosage based on specific production goals, whether for overall health or premium meat quality.

328 The protective effects of yeast-derived products against coccidiosis are complex, involving both modulation of
 329 gut health and immune system priming. The benefits observed in this study—reduced lesion scores, decreased
 330 oocyst shedding, and enhanced gut morphology (Fig. 3) are likely driven by yeast's bioactive components, namely
 331 mannan oligosaccharides (MOS) and β -glucans. MOS acts as a pathogen-blocking agent by mimicking epithelial
 332 receptors that pathogenic bacteria and parasites target. By binding to pathogens such as *Eimeria*, MOS prevents
 333 their adhesion and colonization, facilitating their removal from the gut (Spring et al., 2015; Che et al., 2021). β -

334 Glucans serve as potent immunomodulators, recognized by Dectin-1 receptors on macrophages and other innate
335 immune cells (Goodridge et al., 2009). This interaction initiates a cascade of immune responses, enhancing cytokine
336 production and priming the host for a more effective response to coccidial infection. Furthermore, β -glucans may
337 induce ‘trained immunity,’ a functional reprogramming of innate immune cells that confers enhanced nonspecific
338 protection against subsequent infections (Xiao et al., 2022). Together, these mechanisms—pathogen exclusion and
339 strengthened immune vigilance—likely account for the marked anticoccidial efficacy observed, particularly at the
340 higher dose (3 g/kg), which presumably provided sufficient bioactive compounds to optimally stimulate these
341 protective pathways.
342

343 5. CONCLUSION

344 The findings of this study demonstrate that dietary yeast supplementation provides significant anticoccidial and
345 meat quality benefits in broilers challenged with coccidiosis. Critically, the comparison of two concentrations offers
346 a novel contribution: supplementation at 3 g/kg (Ty2) provided superior overall anticoccidial efficacy and meat
347 yield, while 1 g/kg (Ty1) excelled at enhancing specific meat quality attributes, such as water-holding capacity.
348 This dose-dependent effect provides crucial guidance for its practical application.

349 While growth performance was maintained, the notable improvements in product quality and animal health
350 highlight the potential of yeast as a natural, effective, and safe alternative to conventional anticoccidial agents. The
351 significance of these results lies in delivering optimized, evidence-based dosage recommendations that can directly
352 contribute to sustainable poultry production by mitigating disease losses and enhancing product value. Future
353 studies should explore the molecular mechanisms behind these differential effects and validate these optimized
354 doses under commercial field conditions.
355

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364

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366

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369

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