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(Original Research)

Evaluation of β-1,3/1,6-glucan Supplementation on Growth Performance, Immune Parameters, and Gut Health of Broiler Chickens Vaccinated with Live Attenuated *Eimeria* spp. Vaccine

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Abstract

Background: Coccidiosis, caused by protozoa of the genus *Eimeria*, remains one of the most economically important poultry diseases. Beta-glucans**,** naturally occurring polysaccharides derived from yeast, possess potent immunomodulatory properties and have been shown to enhance innate and adaptive immune responses. The present study investigated the effects of ß-1,3/1,6-glucan (Polymune®), produced from a novel strain of *Aureobasidium pullulans*, on growth performance, immune function, and intestinal health in broiler chicks vaccinated against coccidiosis. **Methods:** A total of 108 day-old Cobb 500® broiler chicks were assigned to a 2 × 3 factorial design with ß-glucan supplementation at 0%, 0.1%, or 0.3% via drinking water from days 1–14, with vaccinated groups receiving an 18× dose of a live attenuated *Eimeria* vaccine (EVANT®) on day 7. Performance indicators included body weight gain (BWG), feed conversion ratio (FCR), gut morphometry, oocyst per gram (OPG) counts, qPCR of *Eimeria* spp. and heterophil-to-lymphocyte (H/L) ratio. Ileum and liver histological lesions were scored using the "I See Inside" (ISI) methodology. **Results:** On day 14, significant differences in BWG were observed due to vaccination ($P = 0.002$), with no interaction between vaccination and ß-glucan supplementation. Vaccination significantly increased relative liver weight ($P=0.010$), while ß-glucan supplementation had no effect ($P > 0.05$). OPG counts were significantly elevated in all vaccinated groups ($P < 0.0001$), peaking on day 12, while unvaccinated groups remained negative. ß-glucan dosage did not significantly influence OPG counts ($P=0.7771$); however, high-dose supplementation (0.3%) showed a numerical reduction in OPG among vaccinated birds. Birds supplemented with 0.3% ß-glucan without vaccination showed higher ISI scores in ileum (17.0 ± 3) and liver (16.0 ± 3) which is comparable to the vaccinated groups. **Conclusion:** Overall, ß-1,3/1,6-glucan showed mixed effects on immune and gut health parameters, with higher doses potentially contributing to enhanced immune response.

Keywords

Beta glucan, *Eimeria*, Live Coccidiosis Vaccine, Broilers

1. Introduction

Coccidiosis is a parasitic disease affecting poultry, causing significant detriment to the global poultry industry annually. It stands out as one of the most economically impactful diseases in chickens, resulting from parasites belonging to the genus *Eimeria* and *Isospora* within the phylum *Apicomplexa* [1]. Each species of these parasites predominantly targets and invades the avian intestinal tract, causing inflammation to epithelial cells and resulting in a range of clinical manifestations in infected chickens. This disruption allows opportunistic bacteria such as *Clostridium perfringens*, *E. coli*, and *Salmonella* to colonize and proliferate in the damaged gut. These effects encompass necrotic gut lesions, reduced weight gain and feed conversion rates, heightened mortality rates, and increased susceptibility to other pathogens [2].

The management of avian coccidiosis involves the utilization of vaccines and antimicrobial medications such as coccidiostats and antibiotics, contributing to the enhancement of the immune system in birds. However, coccidiosis vaccination typically induces mild intestinal lesions and transient changes in performance in chickens as part of the normal immune response. These post-vaccination reactions are expected during the first and second oocyst cycling periods and are essential for the development of protective immunity. However, excessive reactions may occur if vaccine administration is uneven, oocyst cycling is poorly managed, or concurrent enteric pathogens are present, potentially leading to clinical coccidiosis or necrotic enteritis [3].

Despite decades of usage, coccidiosis vaccine outcomes are variable in practice. Several field trials and reviews have documented vaccination failures. A large-scale field trial involving ~900,000 chicks across three farms found that live vaccination sometimes leads to subclinical coccidiosis, with variations in lesion scores, oocyst shedding, and production index (PI), particularly when environmental or management conditions are suboptimal. Reviews of vaccine trials have noted that many experiments differ widely in design (challenge strain, timing, performance metrics), which makes comparing efficacy difficult and may mask failures or underperformances [4].

Antibiotic Growth Promoters (AGPs) and coccidiostats have also been used extensively to improve health, feed efficiency, and weight gain, thereby enhancing the quality of animal-derived products. Nevertheless, the inappropriate and overly frequent utilization of antimicrobials has resulted in the emergence and proliferation of antimicrobial resistance (AMR), the transmission of resistance factors from animals to humans through the presence of drug residues in meat and eggs, and an alteration in the balance of normal microflora [5]. Predictably, the persistent administration of drugs for addressing *Eimeria* infections creates substantial selection pressure that promotes the development of drug resistance [6].

Consequently, developing and providing functional dietary additives as antibiotic alternatives becomes imperative to reduce disease-related mortality and morbidity, maintain feed efficiency and good gut health status, and enhance immunity in poultry [7]. Proposed substitutes for AGPs encompass a range of options such as vaccines, bacteriophages, feed enzymes, plant extracts, organic or inorganic acids, and pro-, pre-, and symbiotic, among others. These alternatives need to demonstrate effectiveness not only in promoting healthy gut function but also in ensuring optimal performance in poultry. Furthermore, alternative agents should be accessible for farmers to utilize on a commercial scale and should be designed to minimize the risk of fostering bacterial resistance [8].

Prebiotics, dietary compounds that remain undigested by the host when consumed but support beneficial bacteria, are gaining significant attention as a promising candidate offering similar advantages. A strategy to mitigate the spread of foodborne pathogens involves incorporating prebiotics into the diet. When consumed, these prebiotics act as substrates for specific bacteria already present in the poultry gastrointestinal tract (GIT) that exhibit antagonistic effects against pathogens [9, 10]. Nurturing beneficial bacteria in the chicken's GIT triggers preventive mechanisms against pathogens through microbial metabolism. This, in turn, leads to changes in the microbial population of the GIT and subsequently enhances the overall health of chickens, as reflected in altered immune responses [9].

Beta-glucans are polysaccharides composed of D-glucose monomers, forming the structural components of cell walls found in yeast, fungi, algae, and cereal grains. ß-glucans contain three distinct glycosidic linkages ß-(1,3), ß-(1,4), and ß-(1,6). The effectiveness of ß-glucans in modulating the immune system varies due to structural differences and based on their origin [11]. Among these molecules, ß-1,3/1,6-glucan produced by a novel strain *Aureobasidium pullulans* has demonstrated the ability to enhance immune functions, particularly beneficial for growing chicks [12]. Acting as an immunomodulator, it may support the development of a healthy immune system.

Despite these known issues, there is limited data on how supplementing immunomodulatory compounds, such as ß-1,3/1,6-glucans, may mitigate vaccine failure in live *Eimeria* vaccination programs and extreme coccidiosis conditions. Although the immunomodulatory effects of ß-glucans have been extensively studied in the past, there is currently a lack of comprehensive information regarding the efficacy of the black yeast *Aureobasidium pullulans* in broiler chickens during live coccidiosis vaccination or extreme challenge conditions. The information obtained from this study will offer researchers essential data for optimizing prebiotics as a viable substitute for coccidiostat during live coccidiosis vaccination or field challenge.

2. Materials and Methods

2.1 Ethical Statement

This study was reviewed and approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (UPLB IACUC) under approval reference number UPLB-2024-006 and protocol review number CAFS-2024-005.

2.2 Experimental Birds, House Management, and Diets

A total of one hundred eight, day old broiler chicks (Cobb500™) were acquired from a commercial hatchery (San Miguel Foods, Inc. Hatchery, Lecheria, Calamba City, Laguna). The chicks had a prior vaccination history for Newcastle disease (NCD) and Infectious Bronchitis (HB1 Mass Blen®) administered via spray, and Infectious Bursal Disease (Transmune®) administered subcutaneously (SQ). Upon arrival, the chicks were weighed. The broiler chickens were housed in an open-sided structure equipped with individual pens. Each pen consisted of a double-walled box measuring 76.2 cm x 57.15 cm x 39.37 cm and featured solid walls. To ensure a clean environment for the birds, completely dry rice hulls, disinfected with formalin prills prior to chick placement, were used as litter material. Additionally, plastic mesh sidings were installed around the pens to safeguard the birds from predators. A floor space allocation of 0.30 m^2 (1 ft^2) per broiler was provided, adhering to recommended space requirements. For brooding, the environmental temperature was initially set at 33-35°C, gradually decreasing until it reached 28-30°C by the end of the experiment. The humidity levels were kept at 60-70%. The lighting schedule entailed 23 hours of light and 1 hour of darkness during the initial week, subsequently reducing to 20 hours of light thereafter.

The birds were offered formulated booster (pre-starter) diets manually, and the birds had *ad libitum* access to both water and feed. All treatment pens were offered the same diet for 14 days. The composition of the booster (pre-starter) diet is presented in Table 1.

In this study, the selected ß-glucans, known as Polymune®, constituted a liquid supplement containing ß-1,3/1,6-glucan derived from a novel strain of *Aureobasidium pullulans*. The ß-glucans were incorporated into the drinking water, with a dosage of 1 mL per liter once a day (Day 1-14) for the 0.1% ß-glucan-treated group, while three times (3 mL/L) the said dosage was offered for the 0.3% ß-glucan-treated group as higher dose. To maintain freshness and cleanliness, the drinking solutions were diligently prepared twice every day at AM and PM, following the guidelines provided by the manufacturer. Daily maintenance involved washing the drinkers in each enclosure while avoiding the waterers of infected and uninfected groups from coming into contact and refilling them with the specified treatment consistently at the same time of day.

2.3 Experimental Design

The broiler chicks were placed into different pens under uniform environmental conditions,

Table 1. Ingredients and nutrient composition of the broiler booster (pre-starter) diet.

Item	Booster (Day 1 - 14)
Ingredient (%)	
Yellow Corn	55.24
Soybean Meal US HP	38.71
Coconut oil	2.07
Limestone	1.1
Monodicalcium Phosphate	2.3
Vitamin Premix	0.05
Mineral Premix	0.05
Refined Iodized Salt	0.3
L-Methionine	0.18
Analyses Nutrient Content (%)	
Crude Protein	20.50
Crude Fat	4.90
Crude Fiber	1.93
Ash	6.73
Moisture	11.27

following a completely randomized design in a 2 x 3 factorial arrangement with coccidiosis infection and ß-glucan dosage as factors and pen as the experimental unit. The experiment included two vaccinated treatments, three distinct water treatments, and three replicate pens arranged randomly for each treatment. Each pen housed six birds (n=108).

Treatment groups:

A. **Negative Control:** No ß-glucan treatment and no live coccidiosis vaccination.
B. **Coccidiosis Vaccinated Control:** With coccidiosis live vaccination but without ß-glucan treatment.
C. **0.1% β-glucan-treated Group:** With ß-glucan treatment at 0.1% concentration and no coccidiosis live vaccination
D. **0.1% β-glucan and Coccidiosis Vaccination:** With ß-glucan treatment at 0.1% concentration and coccidiosis live vaccination.
E. **0.3% β-glucan-treated Group:** With ß-glucan treatment at 0.3% concentration and no coccidiosis live vaccination
F. **0.3% β-glucan and Coccidiosis Vaccination:** With ß-glucan treatment at 0.3% concentration and coccidiosis live vaccination.

2.4 Growth Performance

Morbidity and mortality were assessed daily. Each replicate pen underwent weekly evaluations to measure and compute various growth parameters, including broiler body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Body weight gain (BWG) was calculated by subtracting the initial weight at the start of the week from the weight recorded at the end of the week. Feed intake (FI) was

determined by subtracting the remaining feed in the feeder from the amount initially provided on a given day. Finally, the FCR was computed at the end of the week by dividing the total feed consumed by the experimental birds by their total weight gain.

2.5 *Eimeria* spp. Vaccination

On the specified day 7, each bird in the 3 replicate pens for each ß-glucan supplementation level underwent administration of a 1 mL (18x of recommended dose to also replicate clinical coccidiosis challenge) of live attenuated *E. acervulina, E. maxima, E. mitis, E. praecox*, and *E. tenella* vaccine (EVANT®), manually using a syringe into the oral cavity [13, 14]. These designated pens were labeled as the "vaccinated groups." The formulation of the undiluted vaccine used is presented in Table 2.

Table 2. Formulation of each 7 µL (dose) of undiluted vaccine (EVANT®).

Item	Number of sporulated oocysts at the time of blending *in vitro*
Eimeria acervulina (003 strain)	332 – 450
Eimeria maxima (013 strain)	196 - 265
Eimeria mitis (006 strain)	293 - 397
Eimeria praecox (007 strain)	293 - 397
Eimeria tenella (004 strain)	276 – 374

The process of preparing the diluted vaccine suspension included mixing the 7 mL vaccine with 223 mL water and 50 mL solvent, resulting in a combined volume of 280 mL. Initially, the solvent vial was shaken to ensure uniformity, and its contents were then mixed with clean room temperature water into an appropriate container. Subsequently, the vaccine vial was shaken, and its contents were added to the previously prepared solution. To prevent any potential cross-contamination, the non-vaccinated and vaccinated birds were housed in two distinct yet identical pens, ensuring uniformity in terms of pen size, temperature, and humidity conditions.

2.6 Measurement of Relative Weights of Digestive Organs

At the end of the brooding period (14 days), two broilers per replicate were selected based on the average body weight within each group. Subsequently, these chosen birds were weighed and euthanized through alcohol euthanasia. The gastrointestinal tract (GIT) and organs were then carefully excised. Measurements of the small intestine and caeca lengths of individual birds were taken using a ruler. Additionally, the proventriculus, gizzard, and liver of each bird were weighed [15]. The relative weights of these organs were calculated as percentages of the live weight and expressed accordingly.

2.7 Measurement of Relative Weights of Immune Organs

The same 36 broiler chickens (2 per replicate pen) selected for digestive organ measurement were also used for the evaluation of immune organs. The thymus, spleen, and Bursa of Fabricius were carefully extracted from each carcass and individually weighed. The relative weights were calculated by dividing the weight of each organ (in grams) by the BW of the bird (in kilograms) [16].

A bursameter, a flat plastic ruler with eight calibrated holes ranging from narrowest (1) to widest (8), was used to measure the size of the Bursa of Fabricius [17]. The corresponding diameters per bursameter score are shown in Table 3.

Table 3. Bursameter score and corresponding diameters [16].

Score	Diameter (mm)
1	3.50
2	6.50
3	9.50
4	13.00
5	16.00
6	19.00
7	22.50
8	25.50

2.8 Tissue Section Preparation (Histopathology)

On day 14 (seven days post-vaccination), six broiler chickens per treatment group were euthanized by cervical dislocation. Samples of the ileum and liver were collected and fixed in 10% neutral buffered formalin for at least 24 hours. The tissues were processed following standard histological procedures, including dehydration, paraffin embedding, and sectioning into 5 µm-thick slices. Sections were stained with hematoxylin and eosin for microscopic evaluation. Intestinal lesions were assessed by measuring 20 villi per bird under 10× magnification, with 20× and 40× magnifications used to confirm alterations. Liver samples were examined in 10 fields per bird at 10× magnification using an optical microscope (AmScope T120B-5M, California, USA).

Lesions were scored blindly using the I See Inside (ISI) methodology [18] a system currently in the process of patenting (INPI BR 1020150036019). The ISI method is based on assigning a numeric score of alteration to histological and macroscopic findings. An impact factor (IF) ranging from 1 to 3 is defined for each alteration according to its expected effect on organ functional capacity, supported by prior literature and background research. Severe alterations such as necrosis receive the highest IF (3), as they completely compromise cell function. In addition to IF, the extent of each lesion is evaluated based on intensity or observed frequency relative to non-affected organs or tissues, with scores ranging from 0 (absence of lesion or frequency) to 1 (alteration up to 25% of the area or frequency), 2 (alteration affecting 25–50% of the area or frequency), and 3 (alteration affecting more than 50% of the area or frequency). The final ISI index is calculated by multiplying the extent score by the impact factor for each alteration, then summing the values of all alterations per sample. This approach provides a quantitative measure of gut and liver health by accounting for both the severity and biological relevance of observed lesions.

2.9 Heterophil: Lymphocyte Ratio

At 10 and 14 days of age (3- and 7-days post vaccination), fresh blood samples were obtained from two birds housed in each replicate pen to assess the heterophil-to-lymphocyte ratio. During the blood collection procedure, a gauge 23, 1-inch needle syringe was utilized to withdraw 1.5 mL of blood from the wing vein. Subsequently, the blood samples were promptly transferred to 3 mL ethylene diamine tetra acetic acid (EDTA) tubes (Kingmed) to prevent clotting.

A drop of blood was placed onto a slide, creating a single-cell layer blood smear. The smears were air-dried and subsequently stained using the Giemsa staining set. After staining, the slides were rinsed with distilled water and allowed to air-dry. Following preparation, the slides with blood smears were examined under a microscope at high-power magnification (400x). A total of 100 leukocytes, comprising both granular (heterophils) and non-granular (lymphocytes) cells, were counted to

determine the heterophil-to-lymphocyte ratio [19]. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes in these 100 leukocytes.

2.10 Oocyst per Gram (Modified McMaster Fecalysis) and Realtime PCR of *Eimeria* spp.

Fresh fecal samples were collected per experimental unit (pen as the experimental unit) on days 6, 12, 13, and 14. Samples were refrigerated until analysis. For the Modified McMaster egg counting technique, fecal samples were mixed with 26 mL of Sheather's solution. The suspension was transferred into a McMaster chamber using a pipette, and oocysts were counted under a microscope at 10× objective magnification. The total number of oocysts per gram (OPG) was calculated using the following formula: OPG = Total oocyst count × 25. Concurrently, fecal samples from the treatment and control groups were sent to Hipra Diagnostic Laboratory Philippines for molecular detection of *Eimeria* spp. using a polymerase chain reaction (qPCR) developed at the Institute for Animal Health (Compton, UK) to specifically detect *E. acervulina*, *E. maxima, E. mitis, E. praecox and E. tenella* [20]. Genomic DNA from fecal samples were extracted using an automated extraction protocol on the QIAcube system (Qiagen, Hilden, Germany), following the manufacturer's instructions. Quantitative PCR was performed using the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany). PCR cycling conditions consisted of an initial denaturation at 95 °C for 20 s, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Cycle threshold (Ct) values of ≤38.5 cycles were considered positive, while Ct values greater than 38.5 cycles were considered negative.

2.11 Statistical Analysis

Data were analyzed using a completely randomized design in a 2 × 3 factorial arrangement (three ß-glucan levels × two vaccination statuses). Based on historical data for BW ($\sigma = 0.08$ kg), a priori power analysis indicated that this design provides approximately 91% power ($\alpha = 0.05$, two-sided) to detect a 5% difference in BW between vaccination treatments. Growth performance, organ weights, H/L ratio, and oocyst per gram (OPG) counts were analyzed by two-way ANOVA using the General Linear Model (GLM) procedure in SAS® Studio (SAS Institute Inc., Cary, NC, USA). Post hoc comparisons were conducted using Tukey's HSD test when significant effects were detected. Statistical significance was set at $P < 0.05$, and actual P-values were reported where applicable. For histopathological evaluations, data were expressed as mean ± standard error of the mean (SEM). Normality was assessed using the Shapiro–Wilk test. Two-way ANOVA followed by Tukey's test was performed using GraphPad Prism version 8.0.1 (GraphPad Software, Boston, MA, USA). On graphs and tables, error bars represent SEM. Different superscripts or asterisks indicate significant differences between groups, as detailed in the figure or table legends.

3. Results

3.1 Growth Performance

3.1.1 Body Weight

Table 4 presents the BWG of broiler chickens at day 7 (pre-vaccination) and day 14 (7 days post-vaccination), supplemented with different levels of ß -glucans, and subjected to either live coccidiosis vaccination or no vaccination. At day 14, a significant difference in BWG was observed between vaccinated and non-vaccinated birds ($P = 0.002$). No significant differences were detected among ß-glucan levels at either day 7 or day 14 ($P > 0.05$), and no significant interaction between ß-glucan supplementation and vaccination was found ($P = 0.056$).

3.1.2 Feed Conversion Ratio

FCR of broiler chickens on day 7 (pre-vaccination) and day 14 (7 days post-vaccination), both with and without coccidiosis vaccination, and supplemented with ß-glucans at different levels (Table 4). At both day 7 and day 14, no significant differences in FCR were observed among the varying levels of ß-glucan supplementation and vaccination conditions, with no notable variations in their combined effects ($P > 0.05$).

3.1.3 Mortality

Mortality rates of broiler chickens on day 7 (pre-vaccination) and day 14 (7 days post-vaccination), both with and without coccidiosis vaccination, supplemented with ß-glucans at different levels (Table 4). No significant differences in mortality rates were observed among ß-glucan treatment groups on day 7 (pre-vaccination) ($P > 0.05$). Notably, no mortalities were recorded throughout the post-vaccination period (day 7 onwards). There were no signs of diarrhea observed in all treatment and control groups.

Table 4. Body Weight Gain, FCR, and mortality (d 7 & 14) of broilers chickens supplemented with ß-Glucans (0%, 0.1%, 0.3%) under both vaccinated and unvaccinated conditions with live coccidiosis.

	Body Weight Gain (g)			Feed Conversion Ratio (feed / gain)			Mortality Rates (%)		
		Day 7	Day 14		Day 7	Day 14		Day 0-7	Day 8-14
Main Effects									
Beta glucan level	0%	98.54	210.18	0%	1.09	1.03	0%	0.154	0
	0.10%	92.1	193.46	0.10%	1.44	1.29	0.10%	0.772	0
	0.30%	87.77	203.27	0.30%	1.31	0.94	0.30%	0.772	0
Coccidiosis vaccination	with	-	189.02[b]	with	-	1.17	with	-	0
	without	-	215.59[a]	without	-	1	without	-	0
Source of Variation									
Beta glucan level		0.434	0.173		0.138	0.129		0.54	-
Coccidiosis vaccination		-	0.002		-	0.231		-	-
Beta glucan level x Coccidiosis vaccination		-	0.056		-	0.285		-	-
Interactive Effects									
With coccidiosis	0%	-	193.28	0%	-	1.02	0%	-	0
	0.10%	-	192.89	0.10%	-	1.53	0.10%	-	0
	0.30%	-	180.89	0.30%	-	0.97	0.30%	-	0
Without coccidiosis	0%	-	227.09	0%	-	1.05	0%	-	0
	0.10%	-	194.03	0.10%	-	1.05	0.10%	-	0
	0.30%	-	225.64	0.30%	-	0.91	0.30%	-	0

[ab] within the same column, means with different superscripts are significantly differed ($P < 0.05$).
"-"indicates the absence of vaccination at this time point, hence no value is recorded.

3.2 Gut Health and Morphometry

3.2.1 Digestive Organ Weights and Relative Weights

Table 5 present the relative digestive organ morphometry of broiler chickens, comparing those with and without coccidiosis vaccination, and supplemented with ß-glucans at different levels (0%, 0.1%, 0.3%). It specifically summarizes the weights and relative weights of the proventriculus, gizzard, and liver in the broiler chickens, as well as the weights, relative weights, and lengths of the duodenum, jejunum, ileum, and caeca on day 14.

Among the digestive organs measured, statistical analysis revealed a significant difference (P = 0.010) solely in the relative weight of the liver (Table 6). This difference appeared to be primarily driven by the coccidiosis vaccination, with ß-glucan dosage having no statistically significant effect (P > 0.05). In essence, the relative weights of the proventriculus and gizzard were not significantly impacted by either ß-glucan supplementation, coccidiosis vaccination, or the interactive effects of both factors. While not statistically significant, the relative weights of the proventriculus and gizzard tended to be numerically higher in the coccidiosis-vaccinated birds compared to the non-vaccinated groups.

Measurements of the small intestine and caeca showed significant differences, primarily in the relative weights of the duodenum, jejunum, ileum, and the weight of the caeca, with coccidiosis vaccination being the main influencing factor (P < 0.05). Additionally, ileum length was significantly affected by b ß-glucan levels (P = 0.014). Similar to the liver findings, coccidiosis-vaccinated broiler chickens exhibited higher relative weights compared to unchallenged groups.

3.2.2 Histopathology Scores of the Intestine and Liver

The histopathological scores for both the ileum and liver are shown in Figure 1. Panel (A) presents the Total I See Inside (ISI) histological scores for ileum histopathology, while panel (B) shows the ISI scores for liver histopathology. Vaccinated groups supplemented with ß-glucan (0.1% and 0.3%) exhibited significantly higher ISI scores compared to the non-vaccinated and non-supplemented groups, indicating increased histological lesions. The combination of ß-glucan supplementation and vaccination resulted in an additive increase in histological lesions. The ISI lesion scores of the ileum revealed clear differences among treatments. Birds receiving no ß-glucan supplementation and no coccidiosis vaccination exhibited minimal scores (2.0 ± 1). Supplementation with 0.1% ß-glucan alone resulted in a slight increase in ileal lesion scores (3.0 ± 1), whereas the combination of 0.1% ß-glucan with

Table 5. Summary of digestive organ weights and relative weights in broilers chickens supplemented with ß-Glucan (0%, 0.1%, 0.3%) under both vaccinated and unvaccinated conditions with live coccidiosis.

Weights and Relative Weights of Digestive Organs							
		Proventriculus		Gizzard		Liver	
		wt, g	RW, %	wt, g	RW, %	wt, g	RW, %
Main Effects							
Beta glucan level	0%	3.38	0.8941	16.59	4.42	15.81	4.19
	0.1%	2.92	0.8167	16.59	4.61	14.56	4.11
	0.3%	3.23	0.9100	16.81	4.73	14.98	4.15
Coccidiosis vaccination	with	3.05	0.9011	16.36	4.82	15.49	4.51[a]
	without	3.31	0.8461	16.97	4.36	14.54	3.79[b]
Source of Variation							
Beta glucan level		0.079	0.167	0.982	0.595	0.659	0.970
Coccidiosis vaccination		0.120	0.198	0.581	0.079	0.515	0.010
Beta glucan level x Coccidiosis vaccination		0.206	0.689	0.221	0.246	0.343	0.555
Interactive Effects							
With coccidiosis	0%	3.33	0.9266	17.60	4.85	16.77	4.65
	0.1%	2.92	0.8635	15.30	4.54	15.53	4.57
	0.3%	2.90	0.9132	16.18	5.06	14.17	4.31
Without coccidiosis	0%	3.42	0.8616	15.58	3.99	14.85	3.72
	0.1%	2.93	0.7699	17.88	4.68	13.58	3.65
	0.3%	3.57	0.9068	17.43	4.41	15.80	4.00

wt means weight and RW means relative weight.
[ab] within the same column, means with different superscripts are significantly differed ($P < 0.05$).

Table 6. Summary of weights, relative weights, and lengths of gastrointestinal tract of broiler chickens supplemented with ß-Glucan (0%, 0.1%, 0.3%) under both vaccinated and unvaccinated conditions with live coccidiosis.

Weights, Relative Weights, and Lengths of GI tract													
		Duodenum			Jejunum			Ileum			Caeca		
		wt, g	RW, %	L, cm	wt, g	RW, %	L, cm	wt, g	RW, %	L, cm	wt, g	RW, %	L, cm
Main Effects													
	0%	7.7	2.05	19	8.5	2.26	49.08	6.08	1.62	43.01[a]	3.93	1.04	10.88
Beta glucan level	0.10%	7.57	2.11	18,75	8.94	2.49	46.21	5.97	1.64	42.75[a]	3.12	0.86	10.12
	0.30%	7.81	2.2	18.83	8.38	2.36	46.08	4.88	1.38	35.25[b]	3.18	0.89	10.12
Coccidiosis vaccination	with	7.54	2.22[a]	18.5	9.07	2.67[a]	46.92	5.69	1.68[a]	42.31	2.91[b]	0.85	10.22
	without	7.84	2.02[b]	19.22	8.15	2.08[b]	47.33	5.59	1.41[b]	38.37	3.92[a]	1.01	10.53
Source of Variation													
Beta glucan level		0.887	0.427	0.977	0.64	0.178	0.5	0.115	0.169	0.016	0.181	0.366	0.152
Coccidiosis vaccination		0.455	0.041	0.457	0.08	<0.001	0.859	0.843	0.03	0.101	0.014	0.122	0.393
Beta glucan level x Coccidiosis vaccination		0.701	0.936	0.473	0.188	0.294	0.377	0.513	0.324	0.823	0.215	0.11	0.311
Interactive Effects													
	0%	7.7	2.13	17.83	9.58	2.66	50.08	6.12	1.73	44.42	3.65	1	10.42
With coccidiosis	0.10%	7.5	2.2	19	9.3	2.73	43.67	5.67	1.68	44.25	2.12	0.62	9.92
	0.30%	7.42	2.33	18.67	8.32	2.6	47	5.3	1.64	38.25	2.95	0.92	10.33
	0%	7.7	1.96	20.17	7.42	1.85	48.08	6.05	1.5	41.6	4.22	1.07	11.33
Without coccidiosis	0.10%	7.63	2.01	18.5	8.58	2.25	48.75	6.27	1.6	41.25	4.12	1.1	10.33
	0.30%	7.42	2.08	19	8.45	2.13	45.17	4.47	1.13	32.25	3.42	0.86	9.92

wt means weight, RW means relative weight, and L means length.
[ab] within the same column, means with different superscripts are significantly differed ($P < 0.05$).

coccidiosis vaccination markedly increased scores (24.0 ± 5–6). Similarly, birds supplemented with 0.3% ß-glucan without vaccination showed moderate scores (17.0 ± 3), which further increased with vaccination (25.0 ± 2). A comparable ISI score pattern was observed in liver histology. The control group without ß-glucan or vaccination showed minimal hepatic lesions (1.0 ± 1), whereas ß-glucan supplementation combined with vaccination produced pronounced hepatic immune responses (18.0 ± 4 to 19.0 ± 3). Birds receiving ß-glucan without vaccination exhibited lower liver lesion

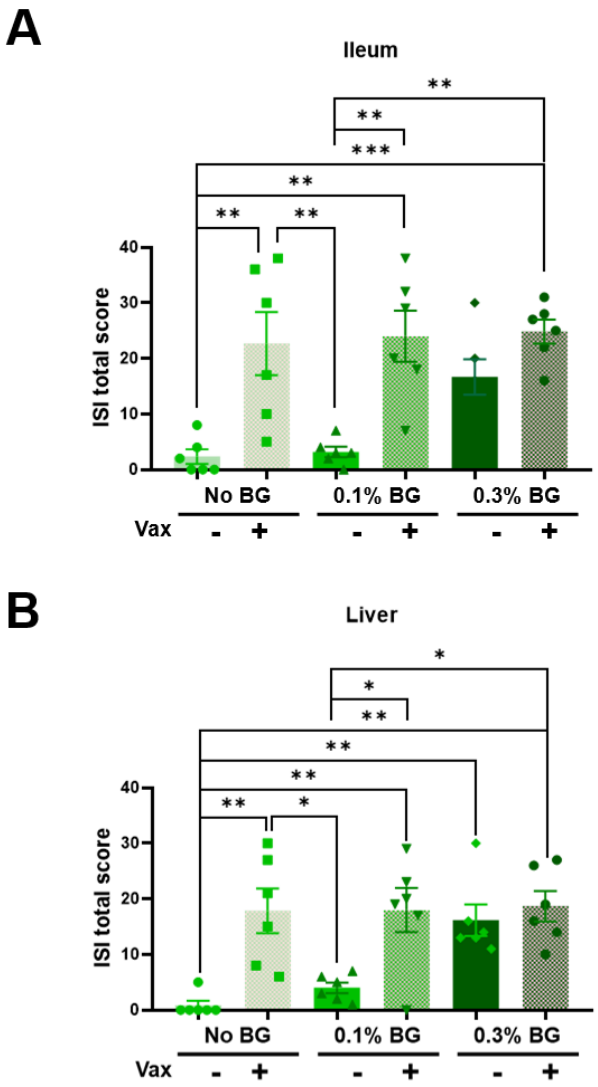

Figure 1. Intestinal and liver histopathology scores of broiler chickens supplemented with ß-glucan (0%, 0.1%, 0.3%) under both vaccinated and unvaccinated conditions with live *Eimeria* spp. Vaccine. Bars represent the mean ± SE, and asterisks indicate statistical significance: () $P < 0.05$, () $P < 0.01$, () $P < 0.001$

scores (4.0 ± 1 for 0.1%; 16.0 ± 3 for 0.3%) compared to their vaccinated counterparts.

3.3 Immune Response

3.3.1 Immune Organ Weights and Relative Weights

Table 7 summarizes the immune response of broilers with and without coccidiosis vaccination, across varying ß-glucan levels (0%, 0.1%, 0.3%). It includes data on the weights and relative weights (compared to body weight) of the thymus, spleen, and Bursa of Fabricius at day 14.

The results revealed significant weight reductions only in the thymus and Bursa of Fabricius of birds vaccinated with coccidiosis ($P < 0.05$). While not statistically significant, the spleen weight also showed a similar decreasing trend. Importantly, there were no significant differences observed in immune organ weight due to varying ß-glucan levels or its interaction with coccidiosis vaccination.

3.3.2 Heterophil to Lymphocyte Ratio (H:L Ratio)

Table 8 summarizes the immune response of broilers with and without coccidiosis vaccination,

Table 7. Summary of immune organ weights and relative weights in broilers chickens supplemented with ß-Glucan (0%, 0.1%, 0.3%) under both vaccinated and unvaccinated conditions with live coccidiosis.

Weights and Relative Weights of Immune Organs								
		Thymus		Spleen		Bursa		
		wt, g	RW, %	wt, g	RW, %	wt, g	RW, %	Bursa-meter
Main Effects								
Beta glucan level	0%	1.62	0.4291	0.4500	0.1203	0.9583	0.2511	4.83
	0.1%	1.52	0.4235	0.4750	0.1332	0.8167	0.2282	4.67
	0.3%	1.45	0.4045	0.4333	0.1215	0.9667	0.2685	4.92
Coccidiosis vaccination	with	1.37[b]	0.4062	0.4278	0.1264	0.77[b]	0.2276	4.56
	without	1.69[a]	0.4319	0.4778	0.1235	1.06[a]	0.2709	5.06
Source of Variation								
Beta glucan level		0.680	0.889	0.768	0.682	0.557	0.621	0.803
Coccidiosis vaccination		0.046	0.558	0.295	0.828	0.032	0.207	0.121
Beta glucan level x Coccidiosis vaccination		0.965	0.946	0.959	0.826	0.704	0.789	0.756
Interactive Effects								
With coccidiosis	0%	1.48	0.4153	0.4167	0.1324	0.78	0.2154	4.67
	0.1%	1.35	0.4023	0.4500	0.1224	0.75	0.2208	4.50
	0.3%	1.27	0.4010	0.4167	0.1287	0.78	0.2467	4.50
Without coccidiosis	0%	1.75	0.4429	0.4833	0.1143	1.13	0.2869	4.83
	0.1%	1.68	0.4447	0.5000	0.1340	0.88	0.2356	5.00
	0.3%	1.63	0.4081	0.4500	0.1182	1.15	0.2902	5.33

wt means weight and RW means relative weight.
[ab] within the same column, means with different superscripts are significantly differed ($P < 0.05$).

Table 8. Heterophil and lymphocyte counts, and heterophil to lymphocyte ratio (d10 and 14) of broiler chickens supplemented with ß-Glucan (0%, 0.1%, 0.3%) under both vaccinated and unvaccinated conditions with live coccidiosis.

Heterophil, Lymphocyte, and Heterophil: Lymphocyte Ratio							
		Day 10			Day 14		
		H	L	H: L	H	L	H: L
Main Effects							
Beta glucan level	0%	25.17	70.33[b]	0.3750	26.25[a]	71.67	0.3676[a]
	0.1%	26.33	71.50[ab]	0.3615	25.28[a]	72.33	0.3550[a]
	0.3%	25.83	72.25[a]	0.3488	24.42[b]	73.08	0.3346[b]
Coccidiosis vaccination	with	26.11	71.50	0.3660	27.00[a]	70.89[b]	0.3816[a]
	without	25.44	71.22	0.3576	23.83[b]	73.83[a]	0.3232[b]
Source of Variation							
Beta glucan level		0.102	0.023	0.059	0.002	0.180	0.005
Coccidiosis vaccination		0.132	0.610	0.335	<0.001	<0.001	<0.001
Beta glucan level x Coccidiosis vaccination		0.183	0.818	0.487	0.1848	0.667	0.234
Interactive Effects							
With coccidiosis	0%	27.17	70.50	0.3860	28.00	70.00	0.4006
	0.1%	26.17	71.83	0.3646	27.50	70.67	0.3897
	0.3%	25.00	72.17	0.3473	25.50	72.00	0.3544
Without coccidiosis	0%	25.50	70.17	0.3639	24.50	73.33	0.3346
	0.1%	25.50	71.17	0.3585	23.67	74.00	0.3202
	0.3%	25.33	72.33	0.3503	23.33	74.17	0.3149

[ab] within the same column, means with different superscripts are significantly differed ($P < 0.05$).

across varying ß-glucan levels (0%, 0.1%, 0.3%). It presents the differential white blood cell counts (heterophils, lymphocytes, and their ratio) at days 10 and 14.

Analysis revealed distinct influences of ß-glucan and coccidiosis vaccination on leukocyte subpopulations depending on the sampling time point. Day 10 data (3 days post-vaccination)

indicated a significant effect of ß-glucan level on lymphocyte counts ($P < 0.05$), with a notable increase observed. Conversely, day 14 (7 days post-challenge), significant differences were observed in heterophil counts and the heterophil-to-lymphocyte (H:L) ratio, mainly due to the ß-glucan level and coccidiosis vaccination ($P < 0.05$). Additionally, a significant difference in lymphocyte counts was noted on this day, mainly due to the effects of the coccidiosis vaccination ($P < 0.001$). There were no significant changes in heterophil counts, lymphocyte counts, or the H:L ratio due to the interactive effects of ß-glucan level and coccidiosis vaccination.

Figure 2 and Table 9 present the summary of oocyst per gram (OPG) counts in broiler chickens supplemented with ß-glucan (0%, 0.1%, 0.3%) under both challenged and unchallenged conditions. Groups A, C, and E (unchallenged) exhibited zero OPG counts across days 6, 12, 13, and 14, while groups B, D, and F (challenged) showed zero counts on day 6 but demonstrated increased OPG counts beginning on day 12.

Table 10 presents the detection of genetic material from five *Eimeria* species *(E. acervulina, E. maxima, E. tenella, E. mitis, E. praecox)* in Treatment B, D, and F on days 12, 13, and 14. The results indicate whether genetic material was detected (POS) or not detected (NEG). When positive, the quantity is categorized as low (+), moderate (++), or large (+++) based on the Ct values. Fecal samples collected on days 12, 13, and 14

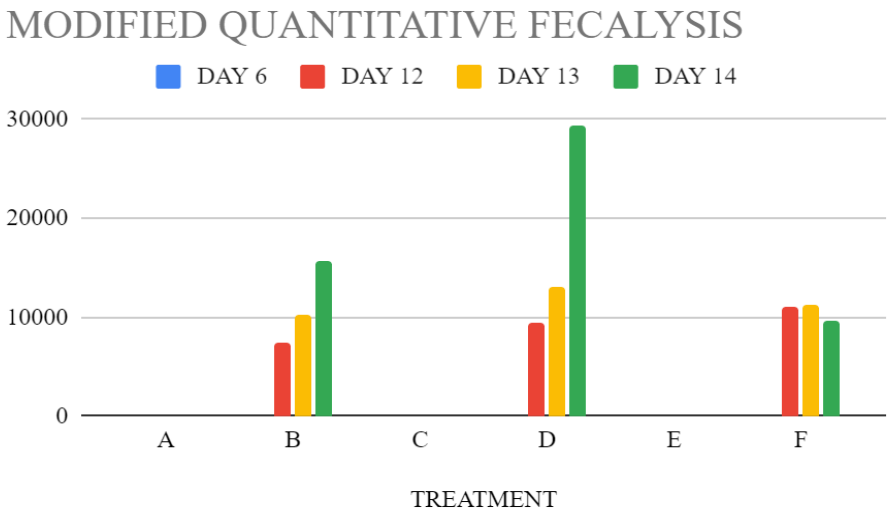

Figure 2. Summary of total mean oocyst counts (OPG) in broiler chickens supplemented with ß-glucan (0%, 1%, 3%) under vaccinated and unvaccinated conditions at days 6, 12, 13, and 14.

Table 9. Effects of coccidiosis vaccination on mean oocyst counts (OPG) in broiler chickens supplemented with ß-glucan (0%, 1%, 3%) on days 12, 13, and 14.

Modified Quantitative McMaster Fecalysis			
	Day 12	Day 13	Day 14
N	6	6	6
Mean	4675	5797	9107
Median	3742	5167	4804
Standard Deviation	5247	6415	11857
Minimum	0	0	0
Maximum	11083	13158	29383

Table 10. Summary of qPCR to detect *Eimeria* spp. vaccinated broiler chickens supplemented with ß-glucan (0%, 1%, 3%) on days 12, 13, and 14 with corresponding average Ct value (n=3).

Eimeria species	Day 12			Day 13			Day 14		
	B	D	F	B	D	F	B	D	F
E. acervulina	+++ (Ct 24.8)	+++ (Ct 24.9)	+++ (Ct 23.5)	+++ (Ct 26.6)	+++ (Ct 26.2)	+++ (Ct 25.4)	+++ (Ct 26.1)	+++ (Ct 27.6)	+++ (Ct 26.6)
E. maxima	NEG	NEG	NEG	+ (Ct 34.6)	++ (Ct 32.9)	++ (Ct 33.0)	++ (Ct 34.5)	++ (Ct 31.3)	++ (Ct 33.7)
E. tenella	NEG	NEG	NEG	+ (Ct 34.3)	NEG	++ (Ct 31.5)	++ (Ct 36.5)	NEG	NEG
E. mitis	+++ (Ct 26.0)	+++ (Ct 26.6)	+++ (Ct 25.3)	+++ (Ct 26.7)	+++ (Ct 25.8)	+++ (Ct 26.8)	+++ (Ct 27.0)	+++ (Ct 29.2)	+++ (Ct 28.1)
E. praecox	+++ (Ct 25.0)	+++ (Ct 26.8)	+++ (Ct 26.4)	+++ (Ct 26.0)	+++ (Ct 24.4)	+++ (Ct 25.9)	+++ (Ct 23.1)	+++ (Ct 24.5)	+++ (Ct 24.9)

Legend
Reference values (Ct): POS < 38.5
NEG: No genetic material of the tested pathogens was detected.
POS (+): A low quantity of genetic material of the tested pathogens was detected.
POS (++): A moderate quantity of genetic material of the tested pathogens was detected.
POS (+++): A large quantity of genetic material of the tested pathogens was detected.

B = Coccidiosis Vaccinated Control
D = 0.1% ß-glucan and Coccidiosis Vaccination
F = 0.3% ß-glucan and Coccidiosis Vaccination

revealed species-specific responses: *E. acervulina* and *E. mitis* maintained high loads across all groups, while *E. maxima* and *E. tenella* exhibited delayed detection. Notably, 0.1% ß-glucan suppressed *E. tenella* but not *E. maxima*, whereas 0.3% ß-glucan correlated with elevated *E. praecox* loads.

Statistical analysis revealed a highly significant main effect of challenging condition ($P < 0.0001$), indicating a strong impact of experimental infection on OPG counts. In contrast, the main effect of ß-glucan dosage level was not significant ($P = 0.2492$), suggesting that different supplementation levels did not significantly influence OPG outcomes. Similarly, the interaction between dosage level and challenging condition was not significant ($P = 0.2492$). Overall, the means for the challenged groups were significantly higher compared to the unchallenged groups, further emphasizing the substantial effect of experimental infection.

4. Discussion

Live *Eimeria* vaccination in broilers has been shown to reduce the severity of intestinal lesions and oocyst shedding, and to improve production indices under field conditions [21]. Pages *et al.* (2025) [22] conducted safety profiles of a live attenuated and two live non-attenuated coccidiosis vaccines administered at overdose (10x) in chickens, following the standard model established in the European Pharmacopeia (Ph. Eur.) monograph. To replicate the extreme challenge condition and vaccination in field (tropical environment), we opted to use attenuated live coccidiosis vaccination (x18 recommended dose). In a study by Wang *et al.* (2019), clinical coccidiosis was induced by giving 20x the regular dose of commercial *Eimeria* vaccines in challenged broiler chicks [14]. In this study, the reduction in BWG observed among *Eimeria* spp. vaccinated broiler chickens confirmed that live coccidiosis vaccination exerted a detrimental effect on early growth performance. This outcome is consistent with previous reports [23], which documented significantly lower BW in coccidiosis-infected chicks compared to unchallenged controls. Similarly, growth depression following vaccination was observed [24], likely attributable to intestinal epithelial damage that impairs nutrient absorption [25, 26]. In addition, studies have suggested that exceeding the recommended doses of coccidiosis vaccines may further impair weight gain by triggering heightened immune activation

[27]. Although live vaccines are generally safe and effective, the increased immune stimulation induced by higher doses demands greater metabolic resources, which may detract from growth performance [28, 29]. Conversely, not all studies align with the present findings. De Sabate *et al.* (2001) reported no significant differences in BW between vaccinated and non-vaccinated birds, a discrepancy that may be explained by compensatory growth mechanisms [30]. Mathis (1999) described how a temporary reduction in growth post-vaccination could be followed by accelerated growth later in the production cycle [31]. Variability in study designs, including broiler strain, rearing conditions, and methods of inducing coccidial infection, as noted by McDougald and Reid (1991), may also contribute to the differences observed across studies [32]. This study did not include a coccidiostat-treated positive control group because the primary objective was to evaluate the interaction effects of ß-glucan supplementation and coccidiosis vaccination. The inclusion of a chemoprophylactic group could have confounded interpretation by altering gut microbiota composition and immune activation of the experimental birds.

The absence of significant differences in FCR among varying levels of ß-glucan supplementation and coccidiosis vaccination, as well as their combined effects, suggests that neither treatment notably impaired feed efficiency during the early stages of broiler development. However, the numerically higher FCR observed with increasing ß-glucan supplementation, particularly at the 0.1% inclusion level, may be attributed to the relatively low energy contribution of ß-glucans and the energy diverted toward immune system activation [11]. While ß-glucans are known to enhance innate immunity [33], they do not appear to significantly improve BWG despite promoting feed intake [34]. Interestingly, increasing the ß-glucan level to 0.3% resulted in a slight improvement in FCR, aligning with observations that yeast cell wall extracts containing ß-glucans may help mitigate performance declines in coccidia-vaccinated birds [35]. The slight numerical increase in FCR observed in vaccinated birds can be explained by the negative effects of coccidiosis vaccination on nutrient absorption or the compensatory increase in feed intake to meet immune system demands [25]. Supporting this, it was reported that vaccination-induced immune responses and mild infection symptoms could temporarily impair feed efficiency [36]. Moreover, the pathophysiological effects of *Eimeria* infection, which disrupt nutrient absorption in the small intestine, likely contribute to reduced growth and worsened FCR [37]. Despite these trends, the differences observed did not reach statistical significance. This agrees with findings that dietary ß-glucan supplementation has no significant effects on BWG and FCR [34]. Similar observations were made showing that ß-glucan supplementation does not adversely affect bird performance regardless of the health status or environmental conditions [38, 39]. In contrast, others documented a significant interaction between ß-glucan supplementation and vaccination [2], while some reported improved feed efficiency with yeast ß-glucan supplementation at later stages of growth [35]. Disparities across studies may be attributed to differences in broiler genetics, housing conditions, ß-glucan type and source, or vaccination protocols. Environmental factors, particularly housing conditions, likely influenced the responses observed in this study. The open-sided housing during the summer season may have exposed broilers to heat stress, a known stressor that reduces feed intake and negatively affects growth performance [40]. Modern broiler strains, genetically selected for rapid growth and high feed intake, are particularly susceptible to heat stress, which disrupts nutrient intake and diverts energy toward thermoregulation [41]. As a result, performance impairments linked to environmental stressors may have masked or compounded the potential effects of ß-glucan supplementation and vaccination on FCR.

No significant differences in mortality rates were observed among the ß-glucan treatment groups during the pre-vaccination period. Mortality during the first week appeared unrelated to ß-glucan supplementation, with environmental factors such as heat stress temperatures reaching up to 36.4°C in the open-sided housing and predation likely contributing to early losses, particularly in the group receiving 0.1% ß-glucans. The absence of mortality across all groups during the second week suggests a potential immunostimulatory effect of ß-glucans, supporting enhanced resistance to infections [42]. Furthermore, despite administering the coccidiosis vaccine at a dosage higher than the recommended level, the absence of mortality demonstrated its margin of safety in birds. This aligns with findings showing that vaccinated

flocks exhibit lower mortality rates compared to their unvaccinated counterparts [43]. Vaccination not only confers protection against coccidiosis but also mitigates the growth depression typically associated with infection, thereby improving overall flock health and survival outcomes [44].

The observed increase in the relative weight of the liver in coccidiosis-vaccinated birds is noteworthy and may be attributed to the immune response triggered by the vaccination. This aligns with reports of a similar increase in liver weight following *E. maxima* infection [45]. However, contrasting findings, which reported a decrease in liver weight with *E. tenella* infection, suggest that the specific *Eimeria* species involved may play a key role in modulating liver response [46, 47]. It is possible that the heightened immune activation following vaccination could lead to temporary hyperplasia in the liver, particularly among Kupffer cells, as observed by Moryani *et al*. (2021), which may explain the increase in liver weight in the vaccinated birds [48].

The increased relative weights of the duodenum, jejunum, ileum, and caeca observed in the coccidiosis-vaccinated birds suggest an immune system response in the GIT. These findings are in line with studies showing an increase in the relative weight and length of the duodenum following vaccination [49], although the effects on the jejunum and ileum were less pronounced. These findings differ from reports of an increase in relative weight, length, and content of intestinal sections with increasing coccidia dose [50]. The increase in relative organ weights could reflect immune system stimulation, where the body allocates resources to the development of immune cells and antibody production. This hypothesis is supported by earlier work highlighting the role of immune activation in influencing intestinal morphology [51]. Interestingly, the increase in relative weight in vaccinated birds may also involve subtle changes in intestinal morphology, such as an increase in villus size, to improve nutrient absorption. The significant effect of ß-glucan supplementation on ileum length suggests that ß-glucans may influence gut development, which may support better intestinal health and immune modulation. However, further research is needed to elucidate the specific mechanisms underlying these changes. In future studies, histomorphometry of the duodenum, jejunum, and ileum is recommended.

The significantly increased ISI histopathological scores observed in the ileum and liver of unvaccinated broilers supplemented with higher dose (0.3%) ß-glucan may be associated with heightened immune activity or other health issues which need further investigation. This finding suggests that a higher ß-glucan dose could elicit a more pronounced local and systemic immune response. Nevertheless, this interpretation requires further validation. To confirm whether the observed liver lesions are related to the elevated ß-glucan supplementation, the determination of serum biomarkers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is recommended. Multiple studies support the histological improvements associated with dietary ß-glucan supplementation, particularly under *Eimeria* challenge or vaccination. At the intestinal level, ß-glucans improve gut morphology by increasing villus height, villus:crypt ratios, and goblet cell numbers, which expands the absorptive surface area and strengthens mucosal defense [52]. Fluctuations in adrenocorticotropic hormone levels under stress [53] can significantly influence the ISI histopathological scores.

The observed reduction in thymus and Bursa of Fabricius weights following coccidiosis vaccination is consistent with previous reports, suggesting an immune system response to the vaccine challenge. Leung *et al*. (2019) similarly noted higher thymus weights in uninfected birds compared to those infected with *Eimeria*, indicating that immune activation following challenge may lead to tissue involution [54]. This is in line with the findings that greater small lymphocyte populations in healthier birds are associated with larger lymphoid organs, suggesting that stress or immune activation may reduce organ size [55]. The significant decrease in Bursa of Fabricius weight further supports this, with others proposing that stress-induced corticosteroid production following infection or vaccination may lead to bursal atrophy [56]. Given the Bursa of Fabricius' critical role as a primary lymphoid organ [5], its reduced weight at day 14 could reflect a transient suppression of immune activity, possibly linked to the immune system's prioritization of responding to the coccidial challenge. This is supported by the decrease in both relative weights and scores of the Bursa of Fabricius after vaccination, although scores across all treatments remained within the healthy range,

suggesting no pathological damage had occurred. Contrary to the present findings, some reported increased bursal development in coccidiosis-infected birds, attributed to the proliferation of *E. tenella* within the Bursa of Fabricius itself [47]. Studies indicate that *E. tenella* may reside in the Bursa of Fabricius, a crucial immune organ in broilers [57, 58]. Infection can trigger an early immune response, as evidenced by increased bursa cell numbers and elevated CD3-T lymphocytes [59]. The inconsistencies in results regarding immune organ weight and coccidiosis may be due to factors such as the severity and dosage of coccidiosis, which can overwhelm the immune system, *Eimeria* species specificity, and the age and health status of the birds. Overall, the monitoring should be extended until harvest date is recommended in future studies. Omara *et al.* (2021) found that 0.1% yeast-derived ß-glucan elevated expression of immune genes in spleen, thymus, and Bursa of Fabricius in broilers challenged with *Eimeria* spp., indicating immune priming and regulation [60]. In vaccinated broilers, yeast cell wall/ß-glucan supplementation has also been shown to increase mucosal IgA, modulate cytokine responses in the ileal mucosa, and reduce oocyst shedding [34][61]. They upregulate tight junction proteins (occludin, claudins) and mucin-2 expression, thereby reducing gut permeability and limiting pathogen translocation [41].

The heterophil-to-lymphocyte (H:L) ratio, a well-established indicator of stress in poultry [62], was evaluated at two time points: day 10 (3 days post-vaccination) and day 14 (7 days post-challenge). Several factors can influence the H:L ratio, including bird age, the severity of infection, and feed additive composition [63, 12]. Heterophils are associated with innate immunity and microbial infection control, whereas lymphocytes primarily mediate antibody production [5]. Studies suggest that elevated doses of coccidiosis vaccines might enhance the immune response in poultry by stimulating stronger cell-mediated immunity, which is critical for combating intracellular pathogens like *Eimeria* [42]. Research also indicates that live attenuated coccidiosis vaccines, which utilize weakened *Eimeria* strains, are designed to be safe and effective even at higher dosages, promoting a protective immune response in poultry with minimal adverse effects [44, 72]. Across all treatment and control groups, the H:L ratios fell outside the generally accepted healthy reference range of 0.5–4.0 [64]. Borges *et al.* (2004) similarly reported H:L values between 0.25 and 0.43 in heat-stressed birds, which may offer a useful comparative reference [65]. The observed increase in lymphocyte counts at day 10 following ß-glucan supplementation is consistent with previous studies showing similar lymphocyte increases in chicks fed with 1,3-1,6 ß-glucan [66]. ß-glucan can mimic pathogen-associated molecules, binding to pattern recognition receptors (PRRs) on white blood cells, and trigger an immune cascade, enhancing overall immune responses [67]. Nonetheless, discrepancies exist, as others found no differences in white blood cell or lymphocyte counts in broilers supplemented with ß-glucan, likely due to variations in ß-glucan type, structure, or dosage [68]. At 7 days post-vaccination, increased heterophil counts and elevated H:L ratios aligned with previous observations in *E. tenella* infections [69], which also noted significant lymphocyte declines post-infection. The rise in heterophils could reflect an intensified immune response against *E. tenella* [69, 70]; however, since heterophils also elevate during stress [62], these results require cautious interpretation. Together, the increased heterophil and lymphocyte counts suggest an active immune response in coccidiosis-vaccinated birds. The elevated H:L ratio further supports this, indicating a dual response targeting both infection and tissue damage [71].

qPCR analysis confirmed that *Eimeria acervulina*, *E. mitis*, and *E. praecox* were consistently detected at high levels (Ct < 27.0) across all vaccinated groups from day 12 through day 14, indicating successful initial cycling of vaccine-derived oocysts. *E. maxima* was detected at low to moderate levels (Ct ≈ 31–34) beginning on day 13, while *E. tenella* was only detected in the vaccinated control and 0.3% ß-glucan group at moderate levels (Ct ≈ 31–36) on day 13. No mortality was observed, confirming that vaccine cycling occurred without excessive pathogenic challenge under the litter floor rearing conditions.

The absence of significant reductions in oocyst counts among broilers supplemented with ß-glucans suggests limited direct anticoccidial activity under the conditions of this study. Although ß-glucans are well-documented for their immunomodulatory effects stimulating both innate and adaptive immune responses in poultry [72], these effects may not necessarily lead to a

reduction in *Eimeria* spp. oocyst shedding. This could be attributed to the nature of immune priming, where ß-glucans enhance immune readiness rather than directly inhibit protozoal replication. The timing of supplementation relative to the challenge, as well as differences in ß-glucan source, purity, and dosage, may also influence the outcome [2]. Future studies should include comparative evaluations of AGPs, including coccidiostats, to fully understand how they differ from ß-1,3/1,6-glucan**.**

In contrast to the present findings, some reported that whole yeast cell products reduced fecal oocyst counts during an *Eimeria* challenge, accompanied by increased macrophage nitric oxide production and proinflammatory cytokine gene expression indicating a stronger immunological activation [73]. However, Ott *et al.* (2018) found that broilers supplemented with ß-glucans shed significantly more oocysts than those given antibiotics, and oocyst counts were not significantly different from control birds, while non-challenged groups showed no detectable oocysts [2]. Taken together, these findings suggest that the mixed effects of ß-glucans may be more produced lesion severity (0.3% dose) in ileum and liver rather than in directly suppressing oocyst shedding. The persistently high oocyst counts in all challenged groups suggest that any immunoprotective effects of ß-glucans were not robust enough to interfere with the parasite's reproductive cycle during the timeframe of this study.

5. Conclusions

In conclusion, the observed significant differences in BWG, gut morphometry and immune responses are linked to the main effects, namely the coccidiosis vaccination status and the level of ß-glucans, acting independently rather than interactively. The absence of a significant interaction between ß-glucans and coccidiosis suggests their effects may be separate. While higher-dose coccidiosis vaccination may have reduced mortality rates and potentially strengthened cell-mediated immunity, as suggested by the observed H:L ratio range, it also had negative consequences. These included decreased BWG, disruption of gut health, and a weakened immune response, evident in lower BWG, gut damage, and stunted immune organ growth. Additionally, vaccination presence was the sole factor associated with significant differences in oocyst shedding, with ß-glucan supplementation showing no direct effect under the study conditions. Meanwhile, dietary ß-glucan inclusion exhibited promise in enhancing immune cell activity by increasing lymphocyte counts and subtly influencing ileum length, suggesting a potential role in gut development.

Availability of Data and Materials

All data are available in this study

Author Contributions

Conceptualization, S.I.C.; Data curation, R.E.A.R., A.L.T., A.G.P.V., K.M.N., S.I.C., and M.J.C.A.; Formal analysis, S.I.C., M.J.C.A., and R.E.A.R.; Writing – Original Draft R.E.A.R.; Writing, R.E.A.R., and S.I.C.

Ethics Approval and Consent to Participate

Not applicable

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Conflict of Interest

The authors declare no conflict of interest.

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