Effects of mixed *Eimeria* challenge on performance, body composition, intestinal health, and expression of nutrient transporter genes of Hy-Line W-36 pullets (0-6 wks of age)

Milan K. Sharma, Guanchen Liu, Dima L. White, Yuguo H. Tompkins, and Woo K. Kim *

Department of Poultry Science, University of Georgia, Athens, GA

ABSTRACT A study was aimed to investigate the effects of mixed *Eimeria* challenge on performance, gastrointestinal health, oxidative stress, inflammation, and expression of nutrient transporter genes of Hy-Line W-36 pullets. A total of 540, 16-d old pullets were randomly allocated into 5 treatment groups with 6 replicate cages, including a nonchallenged control group. A mixed *Eimeria* species solution containing 50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL was prepared and challenged to one group as a high-dose treatment (**High**). The 2-fold serial dilution was done to prepare the medium-high (**Med-High**: 25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina*), the medium-low (**Med-Low**: 12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina*), and the low (**Low**: 6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina*) dose treatments, and these dosages were challenged to 3 remaining groups, respectively. Growth performance, daily feed intake (**FI**), and mortality were calculated from 0-14 d postinfection (**DPI**). Gastrointestinal permeability (**GP**) was measured on 3, 5, 6, 7, and 9 DPI. The result indicated significant linear responses to the *Eimeria* challenge dosage in average body weight and body weight gain ($P < 0.0001$). An interaction between treatment and DPI was observed for FI ($P < 0.0001$). Feed intake significantly dropped from 4 DPI and did not recover until 12 DPI in the challenged groups. The lowest FI for each of the challenged groups was observed on 5 DPI. Gastrointestinal permeability increased linearly, peaking at 5 DPI, and was recovered back to normal by 9 DPI in the challenged groups. Furthermore, gene expression of tight junction proteins was linearly upregulated by increased *Eimeria* dosages. The oxidative status of the pullets was lowered in the challenged groups than the nonchallenged control group, whereas the expression of inflammatory and proinflammatory cytokines was upregulated by *Eimeria* challenge on 6 DPI ($P < 0.05$). The highest mortality was observed in pullets challenged with the High, followed by the Med-High ($P < 0.0001$) on 5 DPI. In summary, the mixed *Eimeria* challenge linearly reduced the growth performance of pullets with an increase in oxidative stress and inflammation. A severe effect of *Eimeria* on gastrointestinal health was observed on 5 or 6 DPI as suggested by GP, tight junction genes, and mortality results. This study indicates that *Eimeria* infection can be a threat to gastrointestinal health related issues in pullets.

Key words: coccidiosis, oxidative stress, gastrointestinal permeability, pullets

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INTRODUCTION

Gastrointestinal health is vital in optimizing the performance of birds, and avian coccidiosis is one of the many factors that have a severe negative impact on birds' health. Avian coccidiosis is a protozoal disease caused by several species of *Eimeria*. These *Eimeria* species attach to the different parts of the intestinal tract and multiply in the epithelial enterocyte, severely damaging the gastrointestinal tract (Conway and McKenzie, 2007; Teng et al., 2020). As a result, it disturbs the digestive physiology mainly with the absorption and digestion of the nutrients, which ultimately has a negative impact on the performance of birds (Conway and McKenzie, 2007; Su et al., 2014; Abdisa et al., 2019; McMullin, 2020 ; Teng et al., 2020). The modern poultry industry raises large numbers of birds in an intensive system, usually at high stocking densities, which provides an ideal condition for *Eimeria* transmission. It has been estimated that coccidiosis alone is responsible for an economic loss of more than 10 billion dollars throughout the

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*Corresponding author: wkkim@uga.edu

world to the poultry industry (Blake et al., 2020 ; Gaghan et al., 2022).

The risk associated with coccidiosis in laying hens is as high as that of broilers at any age groups (Soares et al., 2004; Conway and McKenzie, 2007; McMullin, 2020; Andreopoulou et al., 2022). With the egg industry transitioning from the cage to the cage-free systems, it already became one of the major problems that egg producers will have to face in the near future. However, little is known about the effect of coccidiosis in laying hen pullets and how it affects the performance of the laying hens. The performance of the laying hens at maturity depends to a large extent on the quality of the replacement pullets. There is enough evidence that coccidiosis negatively impacts the gastrointestinal health, immunity, nutrient absorption and digestion, and skeletal health in broilers (Dalloul and Lillehoj, 2006; Hong et al., 2006, Conway and McKenzie, 2007; Su et al., 2014; Abdisa et al., 2019; McMullin, 2020; Akbari Moghaddam Kakhki et al., 2019; Oikeh et al., 2019; Teng et al., 2020; Teng et al., 2021). However, we do not have any knowledge if the laying hen pullets responds the same way as the broilers during coccidiosis as they are selected for different purposes. Previously, Su et al. (2014) observed significant differences between laying hen roosters and broilers in the expression of nutrient transporter and digestive enzymes when challenged with *Eimeria acervulina*, suggesting that the responses to *Eimeria* infections would be different depending on species, age, and maturity of the birds. Thus, there could be main differences in how broilers and layers respond to the coccidiosis. However, till now no research has been done in the laying hen pullets with *Eimeria* infections. Therefore, it is essential to understand the intestinal physiology of laying hen pullets when challenged with different dosages of *Eimeria* mimicking the coccidiosis. Thus, the objective of this study was to investigate the effect of different dosages of mixed *Eimeria* species (*E. maxima, E. tenella, and E. acervulina*) on growth performance, gastrointestinal health, oxidative stress, inflammation, and the expression of nutrient transporter genes in Hy-Line W-36 pullets from 0 to 6 wk of age.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at the Poultry Research Center at the University of Georgia (Athens, GA). The experiment was approved by the Institutional Animal Care and Use Committee of the University of Georgia. A total of 540, 16-d old Hy-Line W-36 pullets were weighed and randomly allocated into 5 treatment groups with 6 replicate cages, including a nonchallenged control group (**Control**). A mixed *Eimeria* species solution containing 50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL was prepared and challenged to one group as a high-dose treatment (**High**). The 2-fold serial dilution was done to prepare the medium-high (**Med-High**: 25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina*), the medium-low (**Med-Low**: 12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina*), and the low (**Low**: 6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina*) dose treatments, and these dosages were inoculated to 3 remaining groups, respectively. The different treatment groups and the number of oocysts used are elaborated in Table 1. The dosages of *Eimeria spp.* oocysts were chosen based on the broiler experiment (Teng et al., 2020). Due to the high mortality in the High treatment group, it was terminated at 14 d postinfection (**DPI**). An experimental mash diet was formulated to meet or exceed the nutritional recommendation of Hy-Line W-36 pullets based on a management guide (Hy- Line International, 2020). The diet composition and formulation are shown in Table 2. The lightning regime for pullets was followed based on the management guidelines for different growth phases (Hy- Line International, 2020). Ad libitum mash feed and water were provided throughout the experiment period. The experiment was conducted from d 16 to 6 wk of age.

Table 1. Treatment groups and the number of *Eimeria* oocysts per treatment.

Treatments[1]	*Eimeria maxima*	*Eimeria tenella*	*Eimeria acervulina*
Control	0	0	0
Low	6,250	6,250	31,250
Med-Low	12,500	12,500	62,500
Med-High	25,000	25,000	125,000
High	50,000	50,000	250,000

[1]Unit: number of oocystsControl: nonchallenged group; Low: Low dose challenged group; Med-low the medium low challenged group; Med-high: the medium high dose challenged group; High: the high dose challenged group.

Growth Performance and Body Composition

The body weight (**BW**) of the pullets was recorded weekly, and feed intake (**FI**) was measured every day from 0 to 28 DPI. Mortalities were recorded and were adjusted for the feed intake and average daily body weight gain (**BWG**). Body composition of the pullets, including bone mineral density (**BMD**), bone mineral content (**BMC**), tissue weight (**TS**: muscle + fat weights), muscle percentage (**MS%**), and fat percentage (**FAT%**) were measured on 6 and 28 DPI using Dual Energy X-ray Densitometry (**DEXA,** GE Healthcare, Chicago, IL).

Gastrointestinal Permeability and Lesion Scores

Gastrointestinal permeability (**GP**) was measured on 3, 5, 6, 7, and 9 DPI using fluorescein isothiocyanate dextran (**FITC-d;** Teng et al., 2020). Briefly, one bird per cage was gavaged with 1 mL of FITC-d at the rate of 2.2 mg/kg of the body weight, and blood was collected 2 h postgavage. Collected blood was kept in a dark container for one h at room temperature for clotting, and serum was collected by centrifuging at 1,000 × g for

Table 2. Ingredient composition and nutrient content of Hy-Line W-36 pullet diet (0−6 wk of age).

Ingredients	Amount (%)
Corn	61.10
Soybean meal	22.37
Distiller's dried grains with soluble	11.18
Dicalcium phosphate	1.68
Limestone	1.41
Soybean oil	1.00
Salt	0.32
Lysine HCL	0.26
DL-Methionine	0.19
Mineral Mix[1]	0.08
Vitamin premix[2]	0.05
L-Threonine	0.04
Calculated composition	
ME (kcal/kg)	3,000
Crude protein (%)	18.50
Total Calcium (%)	1.00
Available P (%)	0.50
dLys (%)	0.99
dMet (%)	0.48
dTSAA (%)	0.74
dThr (%)	0.66
dTrp (%)	0.19
dArg (%)	1.05
dVal (%)	0.90
dIleu (%)	0.79

[1]Mineral mix provided the following in g/100 g diet: $Ca(H_2PO_4)_2 \cdot H_2O$, 3.62; $CaCO_3$, 1.48; KH_2PO_4,1.00; Na_2SeO_4, 0.0002; $MnSO_4 \cdot H_2O$, 0.035; $FeSO_4 \cdot 7H_2O$, 0.05; $MgSO_4 \cdot 7H_2O$, 0.62; KIO_3, 0.001; NaCl, 0.60; $CuSO_4 \cdot 5H_2O$, 0.008; $ZnCO_3$, 0.015; $CoCl_2 \cdot 6H_2O$, 0.00032; $NaMoO_4 \cdot 2H_2O$, 0.0011; KCl, 0.10; dextrose, 0.40.

[2]Vitamin mix provided the following in mg/100 g diet: thiamine-HCl, 1.5; riboflavin 1.5; nicotinic acid amide 15; folic acid 7.5; pyridoxine-HCl, 1.2; d-biotin 3; vitamin B-12 (source concentration, 0.1%) 2; d-calcium pantothenate4; menadione sodium bisulfite, 1.98; α-tocopherol acetate (source 500,000 IU/g), 22.8; cholecalciferol (source5,000,000 IU/g) 0.09; retinyl palmitate (source 500,000 IU/g), 2.8; ethoxyquin, 13.34; I-inositol, 2.5; dextrose,762.2.

15 min. The standard solution was made by diluting FITC-d in pooled serum from unchallenged pullets of the same age. The concentrations of FITC-d in the serum and standard solution were measured using a plate reader at the wavelength of 485 nm (SpectraMax ABS plus, Softmax Pro 7 software, Molecular devices, San Jose, CA).

Intestinal Lesion scoring was done at 3 different parts of the intestinal tract, duodenum, part of jejunum and ileum, and ceca for *E. acervulina*, *E. maxima*, and *E. tenella* on a scale of 0-4. The lesion scores were based on the density of the lesions, inflammation of the intestinal wall, and the intestinal content (Johnson and Reid, 1970; Teng et al., 2020).

Small Intestinal Histomorphology

One bird per cage was humanely euthanized on 6, 14, and 28 DPI, and approximately 3 cm mid-sections of the duodenum, jejunum, and ileum were collected, rinsed with phosphate buffer saline, and fixed in 10% formalin. The fixed intestinal tissues were then cut into small blocks and were embedded into the paraffin. The paraffin blocks were then cut into 4μm transverse sections. The sections were mounted on microscopic slides and stained by hematoxylin and eosin. The slides were then examined under a compound microscope with a camera (BZ-Z800, Keyence Inc., Itasca, IL), and pictures were taken at 4X magnification. The length of the villus (**VH**) and crypt depth (**CD**), and villus length to crypt depth ratio (**VH:CD**) were measured as described by Sharma et al. (2020).

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Small sections of the mid-jejunum were collected on 6, 14, and 28 DPI, rinsed with phosphate buffer saline, snap-frozen in liquid nitrogen, and stored at -80°C until further processing. A total of 20 to 30 mg of the jejunum tissue was homogenized with QiAzol lysis reagents (Qiagen Inc, Valencia, CA) using a bead beater (Biospec Products, Bartlesville, OK). RNA pellets were extracted, followed by isopropanol precipitation and alcohol wash, and dissolved in 200 μL of nuclease-free water. The concentration and purity of the RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The purity of RNA was verified at an optical density ratio of 260 to 280 nm. The concentration of the RNA was normalized to a concentration of 2 μg/μL, and the RNAs were reverse-transcribed using high-capacity cDNA synthesis reverse transcription kits (Applied Biosystems, Foster City, CA). qRT-PCR was performed in duplicate with SYBR Green Master mix in a Step One thermocycler (Applied Biosystems, Foster City, CA). Gene expression from cDNA was analyzed by qRT-PCR using the $2^{-\Delta\Delta Ct}$ relative quantification method with β-actin as a house keeping gene (Teng et al., 2020). Primers for housekeeping genes and target genes are listed in Table 3.

Antioxidant Status

The concentrations of total glutathione (**GSH**), glutathione disulfide (**GSSG**), and malondialdehyde (**MDA**), and the activities of catalase (**CAT**) and superoxide dismutase (**SOD**) were measured on 6, 14, and 28 DPI from liver samples. Briefly, a liver sample from one bird per cage was collected, snap-frozen in liquid nitrogen, and then kept in -80°C until further processing. Liver samples (70-90 μg) were homogenized in one mL of buffer and were centrifuged as per the manufacturer's instructions (Cayman chemical, GSH, GSSG, CAT, SOD assay kits, Ann Arbor, MI; MDA: BioAssay Systems, Hayward, CA). The concentrations of GSH, GSSG, MDA, CAT, and SOD were corrected for the protein concentration following the procedure of Castro et al. (2020) . Total antioxidant capacity (**TAC**) was measured from the serum on above mentioned time points following the manufacturer's instructions (BioAssay Systems, Hayward, CA).

Statistical Analysis

Statistical analyses for growth performance, body composition, and gut permeability were performed as

Table 3. List of primers used for qPCR of the jejunum tissue samples gene expression of tight junction proteins, immune genes, and nutrient transporter genes.

Gene	Gene full name	Forward primer	Reverse primer
Housekeeping gene			
ß-actin	Beta-actin	CAACACAGTGCTGTCTGGTGGTA	ATCGTACTCCTGCTTGCTGATCC
Tight junction protein genes			
JAM-2	Junctional adhesion molecule 2	AGCCTCAAATGGGATTGGATT	CATCAACTTGCATTCGCTTCA
ZO-2	Zonula occludin 2	GGCAAATCATTGAGCAGGA	ATTGATGGTGGCTGTAAAGAG
CLDN-1	Claudin 1	TGGAGGATGACCAGGTGAAGA	CGAGCCACTCTGTTGCCATA
OCLN	Occludin	ACGGCAGCACCTACCTCAA	GGCGAAGAAGCAGATGAG
MUC-2	Mucin 2	ATGCGATGTTAACACAGGACTC	GTGGAGCACAGCAGACTTTG
Nutrient transporter genes			
$b^{O,+}$ AT	Solute carrier family 7, member 9	TTATCACCGCACCTGAAC	AGCATCTGAAGGTGCATAG
b^{o} AT	Solute carrier family 6, member 19	GGGTTTTGTGTTGGCTTAGGAA	TCCATGGCTCTGGCAGAGATTT
EAAT-3	Excitatory amino acid transporter 3	TGCTGCTTTGGATTCCAGTGT	AGCAATGACTGTAGTGCA GAAGTAATATATG
pepT-1	Peptide transporter 1	CCCCTGAGGAGGATCACTGTT	CAAAAGAGCAGCAGCAACGA
y^{+} LAT-1	y+ L amino acid transporter 1	CACACTATGGGCGCATGCT	ATTGTGCCTGGAGGTGTTGGT
SGLT-1	Sodium glucose transporter 1	GCCATGGCCAGGGCTTA	CAATAACCTGATCTGTGCAC CAGTA
GLUT-5	Glucose transporter 5	TCCTCCTGATCAACCGCAAT	TGTGCCCCGGAGCTTCT
Inflammatory cytokines			
IL-10	Interleukin 10	AGCAGATCAAGGAGACGTTC	ATCAGCAGGTACTCCTCGAT
TNF-α	Tumor necrosis factor alpha	CGTGGTTCGAGTCGCTGTAT	CCGTGCAGGTCGAGGTACT
IFN-γ	Interferon gamma	CACATATCTGAGGAGCTCTATAC	GTTCATTCGCGGCTTTG
IL-1ß	Interleukin 1 beta	TGCCTGCAGAAGAAGCCTCG	GACGGGCTCAAAAACCTCCT

one way ANOVA using the PROC GLM procedure of SAS V. 9.4 (SAS Institute Inc., Cary, NC). In contrast, the Kruskal-Wallis test was used for lesion score and split-plot over time for FI. Orthogonal polynomial contrasts were used to determine the effect of increasing oocyst doses on measured responses. Pen was considered as the experimental unit. A significant level was set at $P \leq 0.05$, and means were separated using Fisher's LSD.

RESULTS

Performance and Body Composition

Increasing the *Eimeria* challenge doses resulted in both linear ($P < 0.0001$) and quadratic ($P < 0.05$) reduction in BW and BWG 0-6, 0-14, and 0-28 DPI (Table 4); however, no significant difference was observed from 7 to 14 DPI and 14-28 DPI (data not shown), indicating that *Eimeria* challenge profoundly affected growth performance during the acute infection period (0−6 DPI). Likewise, BMD, BMC, and tissue weight were linearly decreased with the increase in *Eimeria* oocyst doses on 6 DPI ($P < 0.0001$) and 28 DPI ($P < 0.05$; Table 5). However, no significant differences were observed for muscle percentage and fat percentage of the pullets ($P > 0.05$) on both 6 and 28 DPI, suggesting that *Eimeria* infection reduced bone mineralization and affected muscle and fat formation equally.

An interaction between *Eimeria* dosage and days postinfection (**DPI**) was observed for the average daily FI until 14 DPI ($P < 0.0001$; Figure 1). The average daily FI of pullets started to significantly drop from the fourth DPI, and the amount of decreased feed intake depends on the *Eimeria* dosages. The lowest drop in FI was observed in the pullets inoculated with the low dose of *Eimeria* (**Low**), whereas the most significant

Table 4. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) challenge on growth performance of Hy-Line W-36 pullets.

	0−6 DPI		0−14 DPI		0−28 DPI	
Item[1]	BW (g)	BWG (g)	BW (g)	BWG (g)	BW (g)	BWG (g)
Control	184[a]	55.2[a]	285[a]	156[a]	482[a]	354[a]
Low	166[b]	37.4[b]	254[b]	126[b]	447[b]	319[b]
Med-Low	146[c]	17.6[c]	247[bc]	119[b]	431[b]	303[b]
Med-High	140[cd]	12.9[c]	240[bc]	113[b]	422[b]	295[b]
High[2]	133[d]	5.02[c]	223[c]	114[b]	-	-
SEM	4.35	4.40	10.4	6.45	8.80	8.76
P-Value	<0.0001	<0.0001	0.0049	0.0001	0.0005	0.0006
P-Linear	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001
P-Quadratic	0.0321	0.0437	0.5008	0.0109	0.1514	0.1433

[a-d]Values within columns not sharing the superscripts are significantly different at $P < 0.05$.

[1]Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment(12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the High dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL): BW, body weight (g); BWG, body weight gain (g); DPI, days postinfection.

[2]Due to high mortality in the High treatment groups, it was terminated from 14 DPI.

Table 5. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) challenge on body composition of Hy-Line W-36 pullets.

	6 DPI					28 DPI				
Item[1]	BMD (g/cm^3)	BMC (g)	TS (g)	FAT% (%)	MS% (%)	BMD (g/cm^3)	BMC (g)	TS (g)	FAT% (%)	MS% (%)
Control	0.096[a]	3.30[a]	172[a]	14.5	0.860	0.126[a]	8.48	458[a]	10.6	89.4
Low	0.089[b]	2.05[b]	144[b]	14.9	0.850	0.126[a]	7.87	409[b]	14.3	85.7
Med-Low	0.091[b]	2.27[b]	137[b]	14.8	0.850	0.124[a]	7.52	412[b]	13.7	86.3
Med-High	0.086[bc]	1.90[bc]	120[c]	15.7	0.840	0.117[b]	7.06	382[b]	11.2	88.8
High[2]	0.083[c]	1.63[c]	111[c]	13.8	0.860	-	-	-	-	-
SEM	0.002	0.130	5.51	0.850	0.008	0.002	0.44	14.5	1.10	1.10
P-Value	0.0003	<0.0001	<0.0001	0.6047	0.4998	0.0418	0.1392	0.0130	0.0687	0.0659
P-Linear	<0.0001	<0.0001	<0.0001	0.8025	0.9002	0.0127	0.0237	0.0029	0.8163	0.7938
P-Quadratic	0.7902	0.0079	0.1696	0.2561	0.1464	0.1028	0.8482	0.5061	0.0111	0.0108

[a-c]Values within columns not sharing the superscripts are significantly different at $P < 0.05$.

[1]Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment(12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the High dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL): BMD, bone mineral density (g/cm^3); BMC, bone mineral content (g); TS, tissue (g) = muscle + fat weight; MS: muscle; DPI: days postinfection.

[2]Due to high mortality in the High treatment groups, it was terminated from 14 DPI.

reduction in FI was observed in the pullets inoculated with the high dose of *Eimeria* (**High**). Similarly, the amount of time taken to recover was also dependent on the *Eimeria* dosages. Feed intake was recovered back to normal in the low dose group by 7 DPI, whereas in the high dose treatment group, it did not come back to normal until 12 DPI.

Gastrointestinal Permeability (GP) and Lesion Score

The effect of different dosages of *Eimeria* on GP on 3, 5, 6, 7, and 9 DPI is shown in Figure 2. Gastrointestinal permeability increased linearly in response with the *Eimeria* challenge dosage ($P < 0.05$). The highest concentration of the FITC-d was measured from the pullets inoculated with the high dosage of oocysts. The changes in the GP were detected as early as 3 DPI, and the GP was at its peak on 5 DPI in all pullets inoculated with *Eimeria* oocysts. Gastrointestinal permeability started to gradually decrease from 6 DPI and recovered back to normal by 9 DPI in all pullets challenged with any dosage of *Eimeria*.

The results for the intestinal lesion score are shown in Figure 3. The intestinal lesion scores were only significantly different between the challenged and nonchallenged groups ($P < 0.0001$); however, there was no significant difference between the challenged groups in the upper intestine, mid intestine, and ceca. The results indicated that the high and med-high groups had more severe lesions than the low and med-low groups.

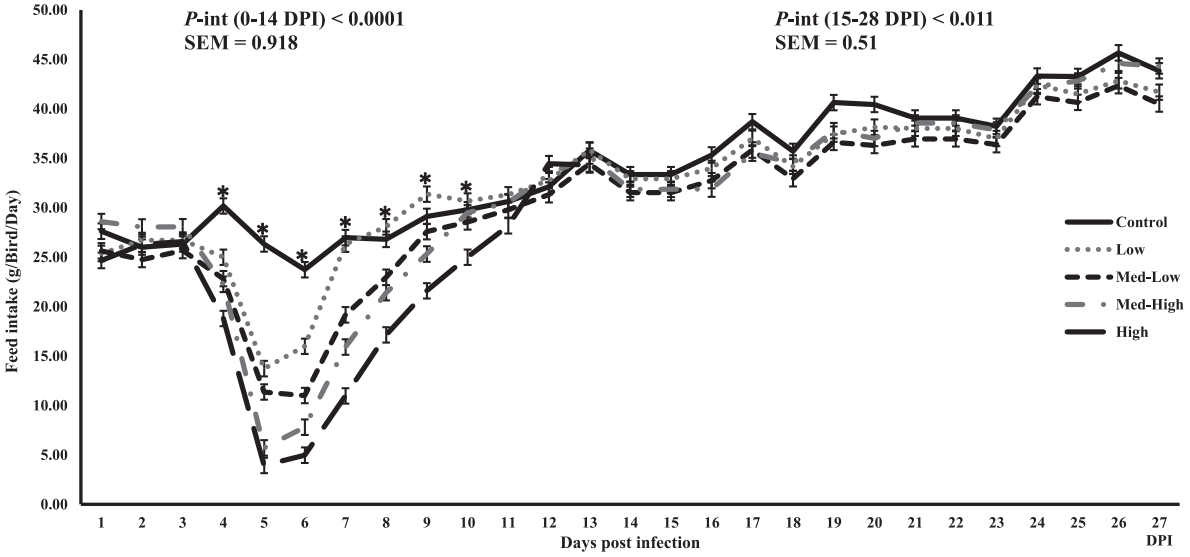


Figure 1. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina*, and *E. tenella*) challenge on average daily feed intake from 0 to 27 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the High dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL). Due to high mortality on the High treatment group, it was terminated from 14 DPI. *P*-int: P value for the interaction between *Eimeria* dosage and days postinfection.

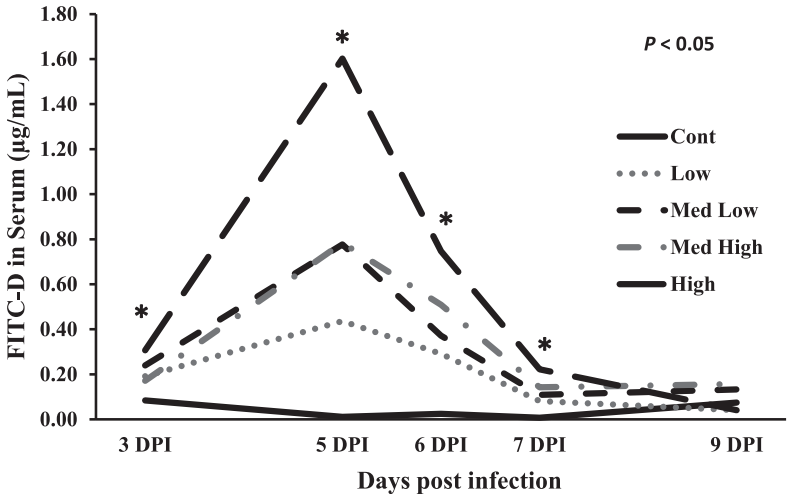


Figure 2. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina*, and *E. tenella*) challenge on gastrointestinal permeability measured by fluorescein isothiocyanate dextran. *Significant linear effects on same days post infection ($P < 0.05$). The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the High dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL).

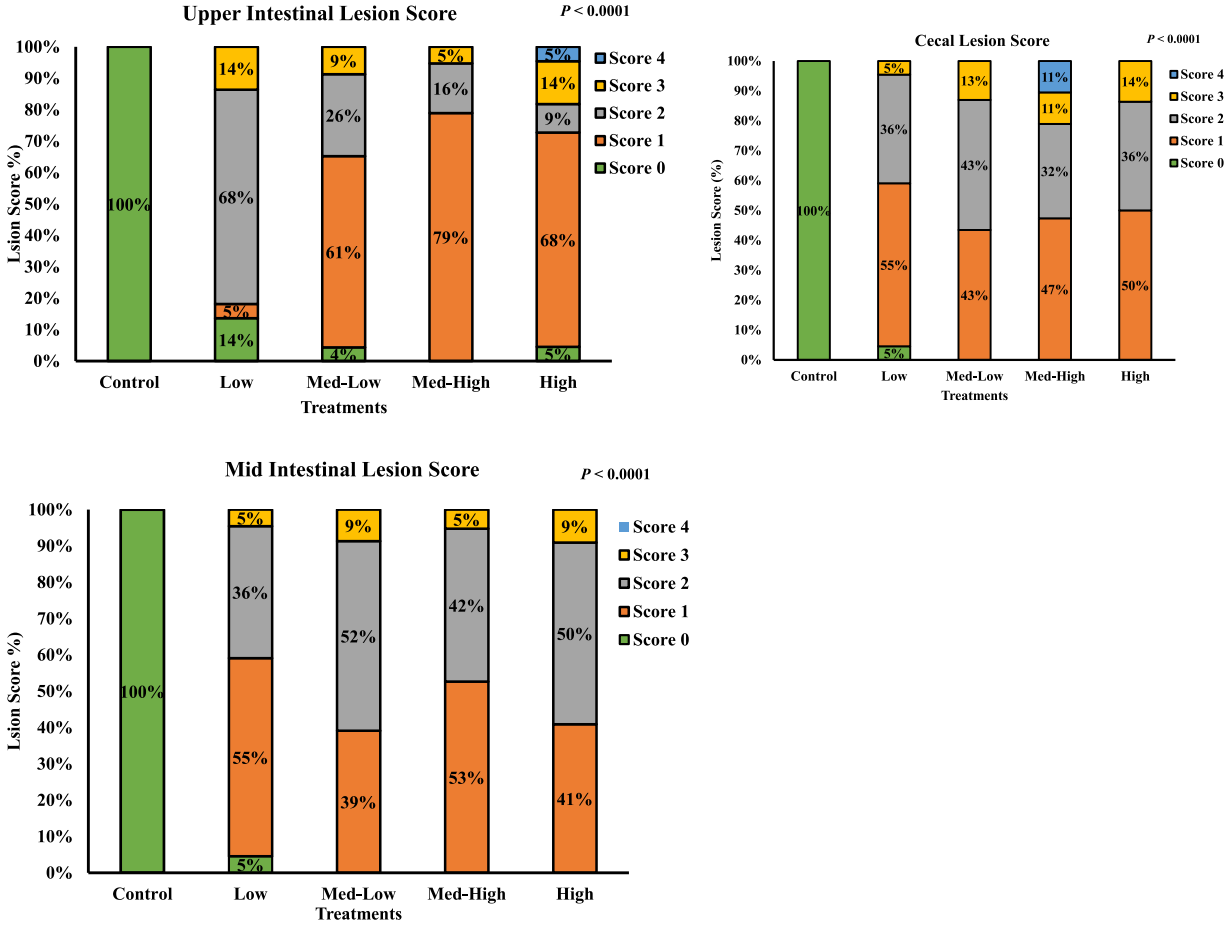


Figure 3. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina*, and *E. tenella*) challenge on lesion score in upper intestine, mid intestine, and ceca of Hy-Line W-36 pullets on 6 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the high-dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL). Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina*, and *E. tenella*) challenge on lesion score in upper intestine, mid intestine, and ceca of Hy-Line W-36 pullets on 6 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the high-dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL).

Table 6. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) challenge on gastrointestinal histomorphology of Hy-Line W-36 pullets.

	6 DPI			14 DPI			28 DPI		
Item[1]	VH (μm)	CD (μm)	VH:CD	VH (μm)	CD (μm)	VH:CD	VH (μm)	CD (μm)	VH:CD
Duodenum									
Control	1433[a]	183[c]	8.00[a]	1597	151[b]	10.7[a]	1758	157[b]	11.3[a]
Low	1188[ab]	339[a]	3.38[b]	1396	333[a]	4.44[b]	1750	241[a]	7.67[b]
Med-Low	1106[b]	318[ab]	3.84[b]	1414	300[a]	4.72[b]	1750	216[a]	8.18[b]
Med-High	908[c]	271[b]	3.49[b]	1601	279[a]	5.76[b]	1708	221[a]	7.80[b]
High[2]	898[c]	308[ab]	2.95[b]	1594	292[a]	5.54[b]	-	-	-
SEM	61.9	23.1	0.430	84.2	19.2	0.45	61.6	13.1	0.42
P-Value	<0.0001	0.0006	<0.0001	0.2235	<0.0001	<0.0001	0.9361	0.0014	<0.0001
P-Linear	<0.0001	0.0187	<0.0001	0.4565	0.0009	<0.0001	0.5913	0.0095	<0.0001
P-Quadratic	0.1531	0.0053	0.0001	0.0862	0.0001	<0.0001	0.7833	0.0068	0.0008
Jejunum									
Control	829[a]	143[b]	6.23[a]	863	119	7.52[a]	1124	131[c]	8.76[a]
Low	737[ab]	260[a]	2.85[b]	813	285	4.06[b]	1223	208[a]	6.04[b]
Med-Low	599[bc]	225[a]	2.73[b]	913	223	4.17[b]	1039	153[bc]	6.81[b]
Med-High	584[bc]	213[a]	3.04[b]	1005	187	5.38[b]	1197	178[ab]	6.73[b]
High[2]	525[c]	235[a]	2.33[b]	1013	199	5.10[b]	-	-	-
SEM	54.6	19.9	0.51	81.3	44.8	0.51	77.6	15.1	
P-Value	0.0076	0.0053	<0.0001	0.3551	0.1458	0.0004	0.3594	0.0112	0.0010
P-Linear	0.0005	0.0359	<0.0001	0.0717	0.6656	0.0431	0.9214	0.2097	0.0087
P-Quadratic	0.5088	0.0321	0.0054	0.7276	0.1076	0.0008	0.7078	0.1025	0.0043
Ileum									
Control	585[a]	121[b]	5.16[a]	599	108[b]	5.58	777[a]	98[b]	8.12[a]
Low	546[a]	191[a]	2.93[b]	639	148[a]	4.53	725[ab]	156[a]	5.04[b]
Med-Low	444[b]	177[a]	2.55[b]	577	136[ab]	4.43	707[ab]	121[ab]	5.95[b]
Med-High	398[b]	189[a]	2.29[b]	636	145[a]	4.38	631[b]	114[b]	5.62[b]
High[2]	386[b]	162[ab]	2.40[b]	614	155[a]	4.00	-	-	-
SEM	31.5	16.8	0.38	35.4	26.7	0.38	27.9	13.3	0.48
P-Value	0.0002	0.0406	<0.0001	0.7120	0.0226	0.0702	0.0160	0.0334	0.0011
P-Linear	<0.0001	0.1375	<0.0001	0.8100	0.0082	0.0102	0.0020	0.8404	0.0074
P-Quadratic	0.3542	0.0132	0.0026	0.9770	0.3056	0.3247	0.6774	0.0231	0.0103

[a-c]Values within columns not sharing the superscripts are significantly different at $P < 0.05$.

[1]Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment(12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the High dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL): CD, crypt depth (μm); DPI, days postinfection; VH, villus height (μm); VH:CD, villus height to crypt depth ratio.

[2]Due to high mortality on the High treatment group, it was terminated from 14 DPI.

Small Intestinal Histomorphology

The effect of different inoculation dosages of *Eimeria* oocysts on morphological characteristics of small intestinal tissues (duodenum, jejunum, and ileum) is shown in Table 6. Increased in inoculation dosages linearly decreased the VH of the duodenum, jejunum, and ileum on 6 DPI ($P < 0.001$). In addition, CD and VH:CD decreased both linearly and quadratically with an increase in the inoculum dosages ($P < 0.05$) on 6 DPI. Villus heights for the duodenum and jejunum were not significantly different on 14 and 28 DPI; however, VH for the ileum was significantly different on 28 DPI ($P = 0.016$). Crypt depth of the duodenum, jejunum, and ileum decreased linearly with increased inoculum dosages ($P < 0.05$) on 14 and 28 DPI. The villus height to crypt depth ratio was linearly and quadratically decreased with increased inoculation dosages on both 14 and 28 DPI ($P < 0.05$).

Gene Expression

Tight Junction Proteins: Changes in the gene expression of tight junction proteins and mucin in pullets challenged with mixed *Eimeria* species are shown in Figure 4. Claudin (**CLDN-1**), Junctional adhesion molecule-2 (**JAM-2**), and Zonula occludin (**ZO-2**) were linearly upregulated, whereas mucin (**MUC-2**) was linearly downregulated with an increase in the *Eimeria* inoculation dosages ($P < 0.05$) on 6 DPI. However, no significant difference was observed for occludin on 6 DPI (**OCLN**; $P > 0.05$). Mucin-2 levels in the Low and Med-Low groups were significantly lower than the Control on 14 DPI ($P = 0.0188$), and *Eimeria* infection quadratically reduced its expression ($P = 0.0815$). However, no significant differences were observed for tight junction proteins on 14 DPI ($P > 0.05$). On 28 DPI, OCLN ($P = 0.0313$), JAM-2 ($P = 0.097$), and ZO-2 ($P = 0.0226$) were significantly upregulated by *Eimeria* infection, and OCLN expression increased linearly with an increase in the *Eimeria* dosages ($P = 0.0102$) on 28 DPI.

Nutrient Transporter Genes: Changes in the expression of nutrient transporter genes in pullets challenged with mixed *Eimeria* species are shown in Figure 5. Expression of amino acid transporters, $b^{O,+}$ AT, b^{O} AT, EAAT-3, and PepT-1 were linearly and quadratically downregulated, whereas y^{+} LAT-1 was linearly upregulated in the pullets challenged with the mixed *Eimeria* species on 6 DPI ($P < 0.05$). However, the expression was not significantly different on 14 and 28 DPI ($P > 0.05$). Expression of glucose transporter GLUT-5 and

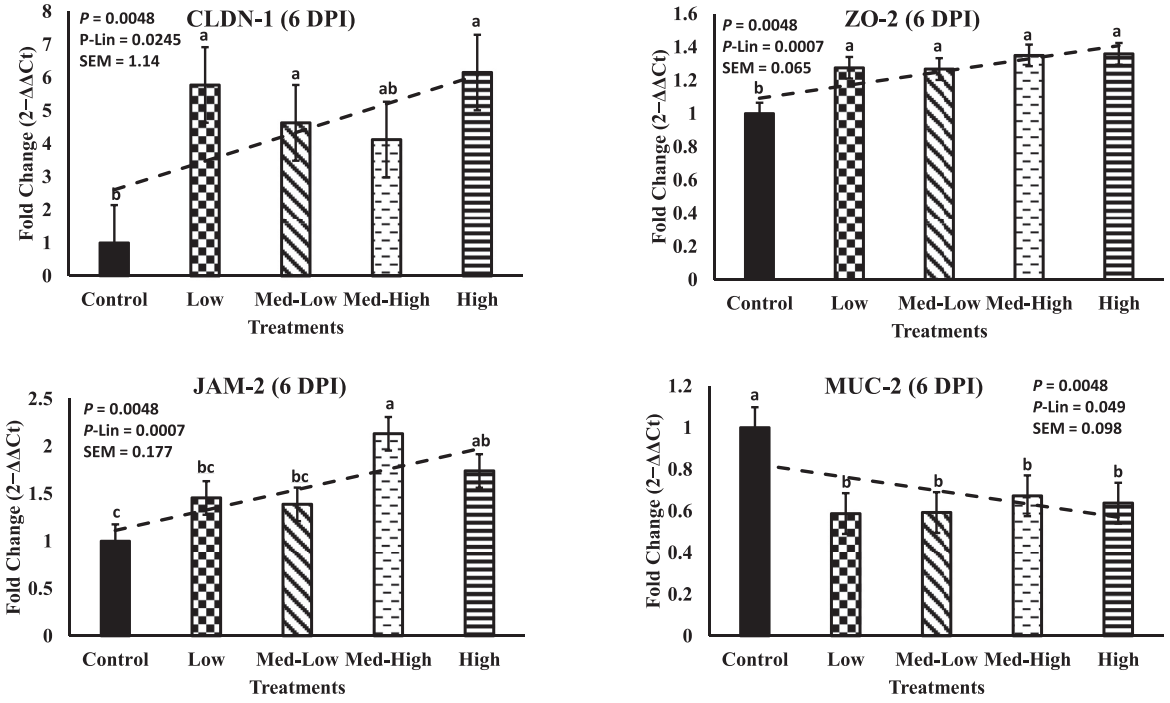


Figure 4. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) challenge on the expression of tight junction protein genes (CLDN-1, claudin 1; ZO-2, Zonula occludin 2 and JAM-2, junctional adhesion molecule 2) and Mucin 2 (MUC-2) of Hy-Line W-36 pullets on 6, 14 and 28 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the high-dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL). Due to high mortality in the high-treatment group, it was terminated from 14 DPI.

SGLT-1 were not significantly different on 6 DPI ($P < 0.05$); however, GLUT-5 was quadratically downregulated on 14 DPI ($P = 0.0487$).

Inflammatory Cytokines: Changes in the expression of inflammatory cytokine genes in pullets challenged with mixed *Eimeria* species are shown in Figure 6. Expression of inflammatory cytokines including interferon-gamma (**IFN- γ**) increased both linearly ($P < 0.0001$) and quadratically ($P = 0.0027$), whereas interleukin 10 (**IL-10**) was cubically upregulated in challenged pullets ($P = 0.0282$). At the same time, tumour necrosis factor-alpha (**TNF-α**) expression was quadratically downregulated in pullets challenged with *Eimeria* species ($P = 0.0122$; P-Quad $= 0.0033$). Expression of interleukin-1 beta (**IL-1β**) was significantly upregulated in the Med-High treatment group compared to the Control and other challenge groups ($P = 0.0007$).

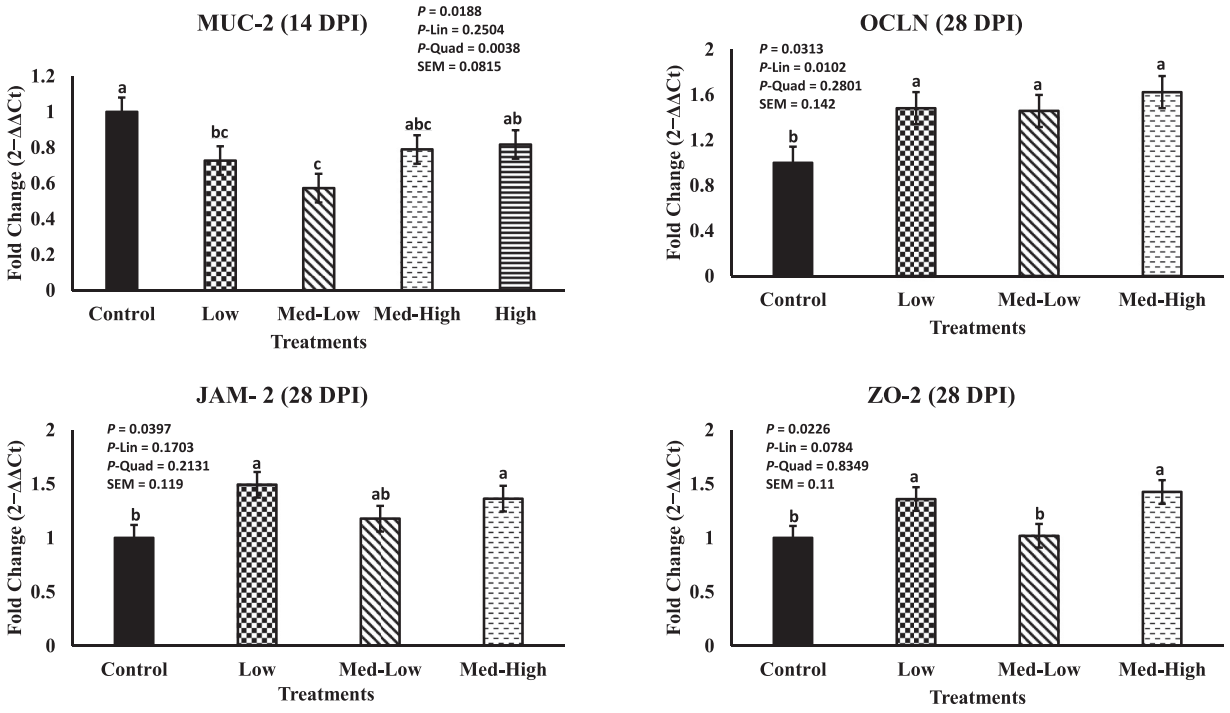
Oxidative Stress

The effect of different inoculation dosages of *Eimeria* oocysts on oxidative stress is shown in Figure 7. Increased in the challenge dosages of *Eimeria spp.* linearly decreased the concentration of the GSH and GSSG ($P < 0.05$) on 6 DPI. The concentration of MDA and SOD linearly increased with the increase in challenge dosages ($P < 0.05$) on 6 DPI. The total antioxidant capacity of pullets decreased quadratically decreased with an increase in challenge dosages ($P < 0.05$) on 6 DPI. No significant differences were observed on 14 and 28 DPI on any of the measured antioxidant markers (data not shown).

DISCUSSION

In the current study, BW, BWG, tissue weight, BMC, and BMD of pullets challenged with mixed *Eimeria* species decreased both linearly and quadratically with an increase in the challenge dosages, which agrees with the previous study by Teng et al. (2020). The reduced performance and body composition of the birds can be explained due to the damage to the intestinal enterocytes, severely affecting nutrient absorption and utilization. Damage to the intestinal enterocytes reduces the secretion of brush border enzymes such as sucrase, isomaltose, or aminopeptidases, affecting the digestion of carbohydrates and proteins (Paris and Wong, 2013; Su et al., 2014) In addition, it has been postulated that *Eimeria* oocysts might compete with the host cells for nutrients in the GI tract to proliferate in host cells (Wang et al., 2018; Teng et al., 2020; Poudel et al., 2021).

Feed intake in pullets challenged with the *Eimeria* oocysts started to drop from 4 DPI in the present study. The degree of the feed intake reduction was dependent on the severity of the challenge, and it did not recover until 12 DPI in the high *Eimeria* challenge group (High) in the present study. The change in the gastrointestinal



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permeability was observed as early as 3 DPI in pullets challenged with *Eimeria* oocysts and was at their peak on 5 DPI. The difference in the FI and the gastrointestinal permeability seemed to be related to *Eimeria*'s life cycle, as explained by Teng et al. (2020). Briefly, when the birds ingest sporulated oocysts, sporozoites are then released into the GI tract and then enter the intestinal enterocytes. These sporozoites then become trophozoites within 12 h to 48 h postinfection and propagate asexually to form schizonts. Once schizonts become mature, they rupture and release merozoites into the intestine on 3 DPI. This release of the merozoites into the intestine brings about changes in the gastrointestinal permeability, and from there onwards, feed intake starts decreasing. These first-generation merozoites again enter the intestinal enterocytes and release the second-generation merozoites, which causes severe damage to the intestinal tract, which is why we have seen the peak in the gastrointestinal permeability as well as severe drop in FI on 5 DPI. In the current study, an increase in the *Eimeria* oocysts linearly increased the gastrointestinal permeability on 3, 5, 6, and 7 DPI, which might be due to the greater number of sporozoites at the beginning may reproduce large numbers of schizonts and merozoites causing severe damage by rupturing the enterocytes (Teng et al., 2020). In addition, we observed high mortality on 5 DPI in the high dose treatment group, which might also be related to the release of second-generation merozoites severely damaging the intestinal tract, which resulted in high mortality. There were no significant differences between the challenge groups for the lesion scoring on 6 DPI in the present study, which might be attributed to the high mortality (a lesion score of 4) on 5 DPI for the high-dose treatment group. Because the lesion scores were not measured in dead birds which might have been higher lesion scores compared to the live ones, the lesion scores in the High group might have been underestimated in the present study.

In the current study, the villus height and villus height to crypt depth ratio decreased both linearly and quadratically with an increase in the challenge dose. In contrast, crypt depth increased by *Eimeria* challenge in all the measured intestinal sections. The reduction in the villus height might be due to the severe damage caused by the release of the merozoites or as a defence mechanism to limit the infection by shedding the enterocytes invaded by *Eimeria* spp. (Cliffe et al., 2005 ; Clevers, 2013; Teng et al., 2020). Deeper crypts were observed in the pullets challenged with the *Eimeria* oocysts, resulting in the lower VH:CD ratio. The deeper crypt might indicate the higher epithelial turnover as a result of infection and defence mechanisms (Wang et al., 2018). Intestinal crypts contain the stem cells which proliferate to compensate for the damage caused by the pathogens or their toxins and, in our case, to make up the loss of intestinal villus (Awad et al., 2009).

The intestinal epithelium plays an important role in protecting the gastrointestinal tract from pathogens (Awad et al., 2017; Chaudhari et al., 2020). The gastrointestinal tract is covered with mucosal layers and is organized into 2 layers: the outer mucosal layer, which acts as the first line of defence against pathogens, and the inner layer, which is tightly bound to the epithelial cells, prevents pathogens from entering the epithelial cells, and is regulated by the expression of Mucin-2 (MUC-2; Johansson et al., 2011; Lee et al., 2017). In addition, these epithelial cells are tightly bound together by intracellular tight junctional proteins, which hold the

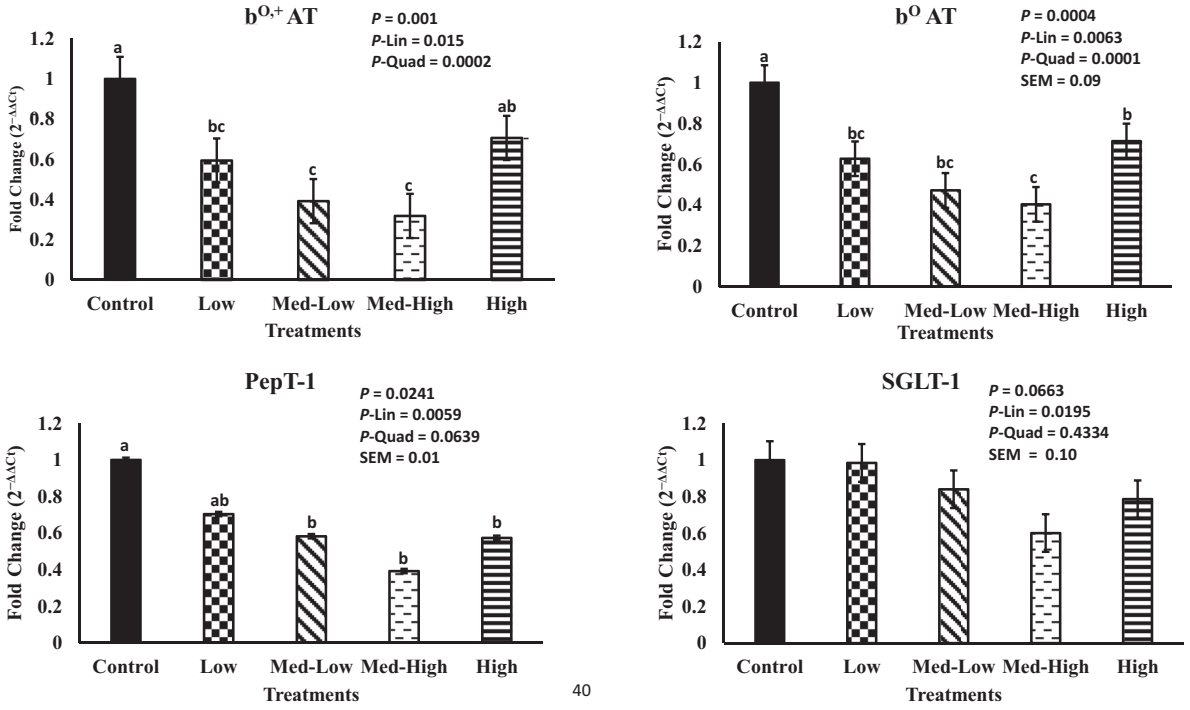
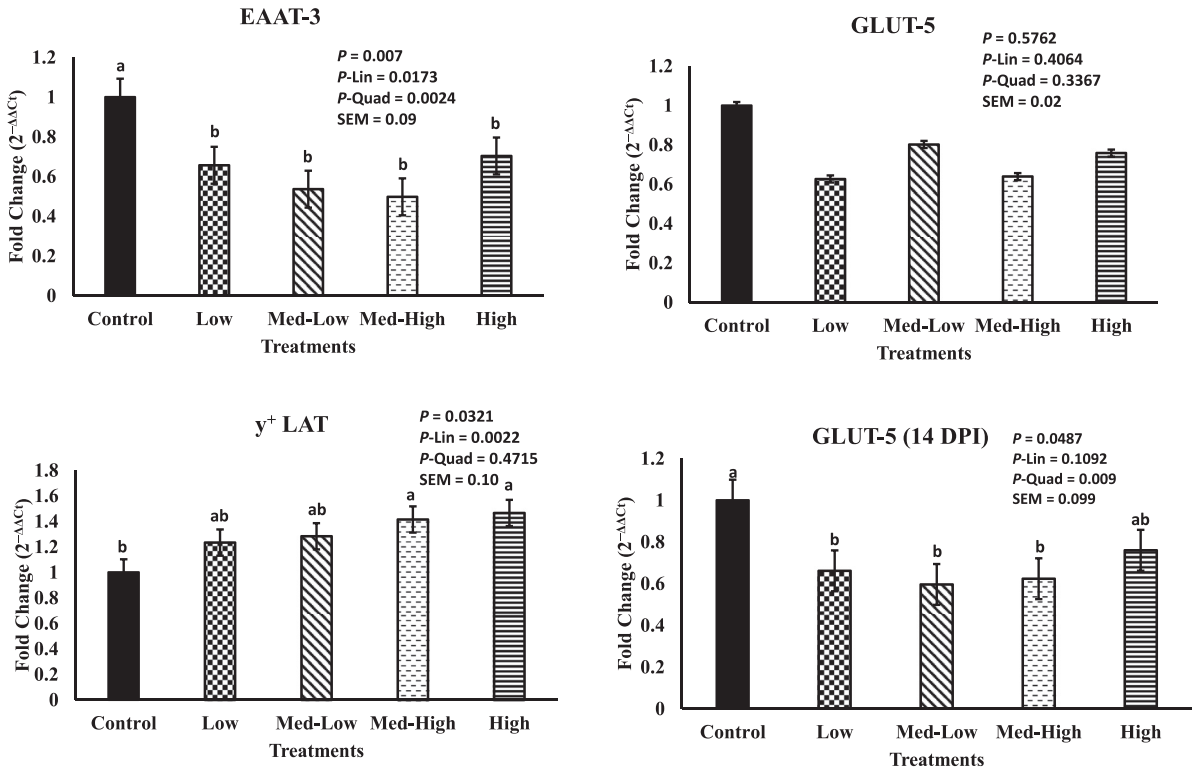


Figure 5. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina*, and *E. tenella*) challenge on the expression of nutrient transporter genes ($b^{o,+}$AT, solute carrier family 6, member 9; b^{o}AT, solute carrier family 6, member 19; pepT-1, Peptide transporter-1; SGLT-1, Sodium glucose transporter-1; EAAT-3, excitatory amino acid transporter 3; GLUT-5, glucose transporter-5; and y+ LAT, y+ L amino acid transporter-1) of Hy-Line W-36 pullets on 6 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the High dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL). Due to high mortality in the high treatment groups, it was terminated from 14 DPI.

enterocyte cells together, forming a protective barrier. The major components of tight junction proteins include occludin, claudin, junctional adhesion protein, and zonula occludin proteins (Ulluwishewa et al., 2011; Awad et al., 2017). In the present study, CLDN-1, which is considered as the backbone of tight junction, JAM protein-2 (JAM-2), which regulates the epithelial barrier function; and Zonula occluding-2 (ZO-2), which governs the tight epithelial junctions (Awad et al., 2017), were all linearly upregulated with an increase in challenge dosages. This upregulation of tight junction proteins was brought by the damage to the epithelial enterocytes by merozoites and acute inflammation of the intestinal tract or oxidative stress during the coccidial infection to rebuild the integrity of the intestine (Awad et al., 2017). Mucin-2, which plays a vital role in establishing the mucus layer of the intestine, was linearly downregulated with increased *Eimeria* dosage. During the coccidiosis, MUC-2 expression was downregulated by *Eimeria*, preventing the mucus layer replenishment and increasing the chances of further infection (Lillehoj et al., 2004).

The small intestine is the major site for the absorption of most of the nutrients. Specific nutrient transporters located at the epithelial brush border membrane facilitate the absorption of most of the free amino acids, short peptides, monosaccharides, and polysaccharides from the intestinal lumen to the enterocytes. The final digestion of these nutrients is catalysed by specific enzymes and is again transferred to the portal blood system via the transporters located at the basolateral membrane (Bröer, 2008; Su et al., 2014). In the current study, linear and quadratic downregulation of several brush border nutrient transporters ($b^{O,+}$ AT, b^{O} AT, EAAT-3, and PepT1) was observed, whereas basolateral transporter (y^{+} LAT-1) was linearly upregulated. These results were similar to the previous study by Su et al. (2014) where 21-d old male roosters were challenged with the *E. acervulina* at the rate of 200,000 oocysts/bird. Downregulation of these brush border amino acid transporters reduces the uptake of amino acids from the intestinal lumen to the epithelial enterocytes, delaying the mucosal growth and repair (Fan et al., 2004; Parisi et al., 2015). EAAT-3 for L-glutamate, which serves as the metabolic fuel for the intestinal enterocytes (Fan et al., 2004; Su et al., 2014), was downregulated, limiting the uptake of this amino acid during the *Eimeria* challenge condition in the present study. The combined effect of downregulation of amino acid transporters decreases the intracellular concentration of the essential amino acids in the intestinal enterocytes. This will eventually starve the cells and shut down the protein synthesis, leading to cell death. Paris and Wong (2013) proposed this cell death as the defence mechanism to restrict the multiplication of *Eimeria Spp.*

Proinflammatory cytokines including IL-1β, IFN- γ, and TNF-α are mainly produced by the activated macrophages in response to the bacterial or protozoal infection (Giansanti et al., 2007). As a result, they



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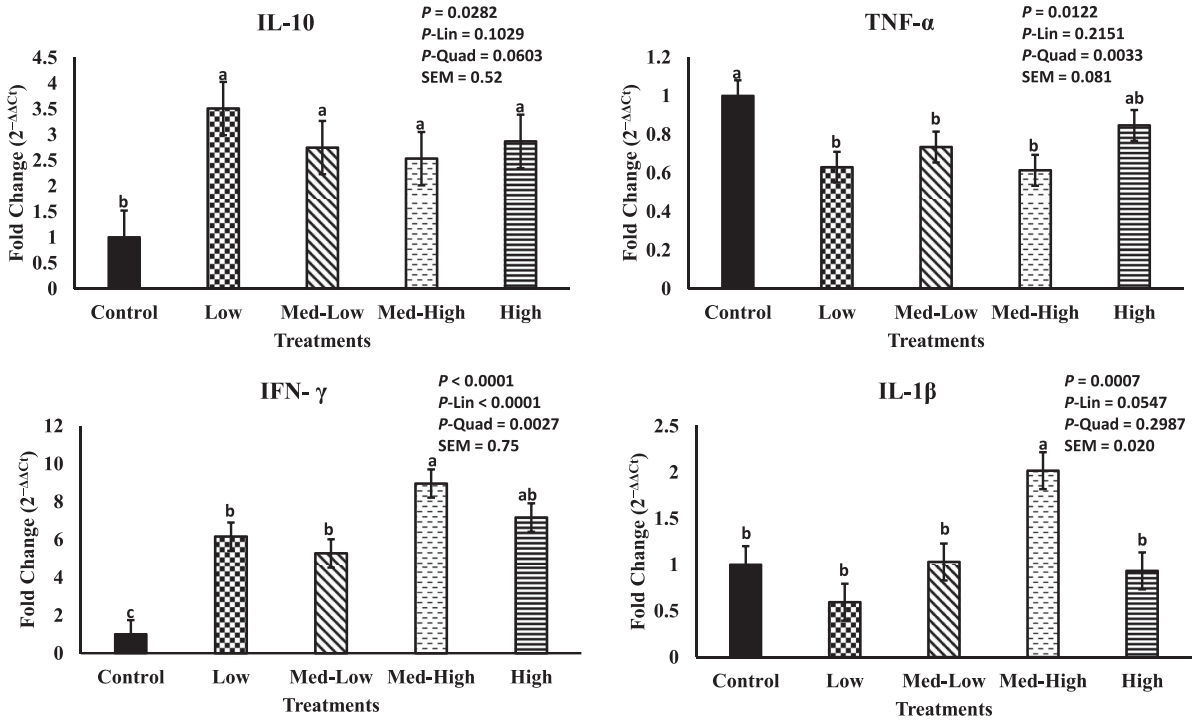

Figure 6. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) challenge on the expression of proinflammatory and inflammatory cytokines genes (IL-10, interleukin 10; TNF-α, Tumor necrosis factor alpha; IFN- γ, interferon gamma; IL-1β, interleukin 1 beta) of Hy-Line W-36 pullets on 6 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the high-dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL). Due to high mortality in the high treatment groups, it was terminated from 14 DPI.

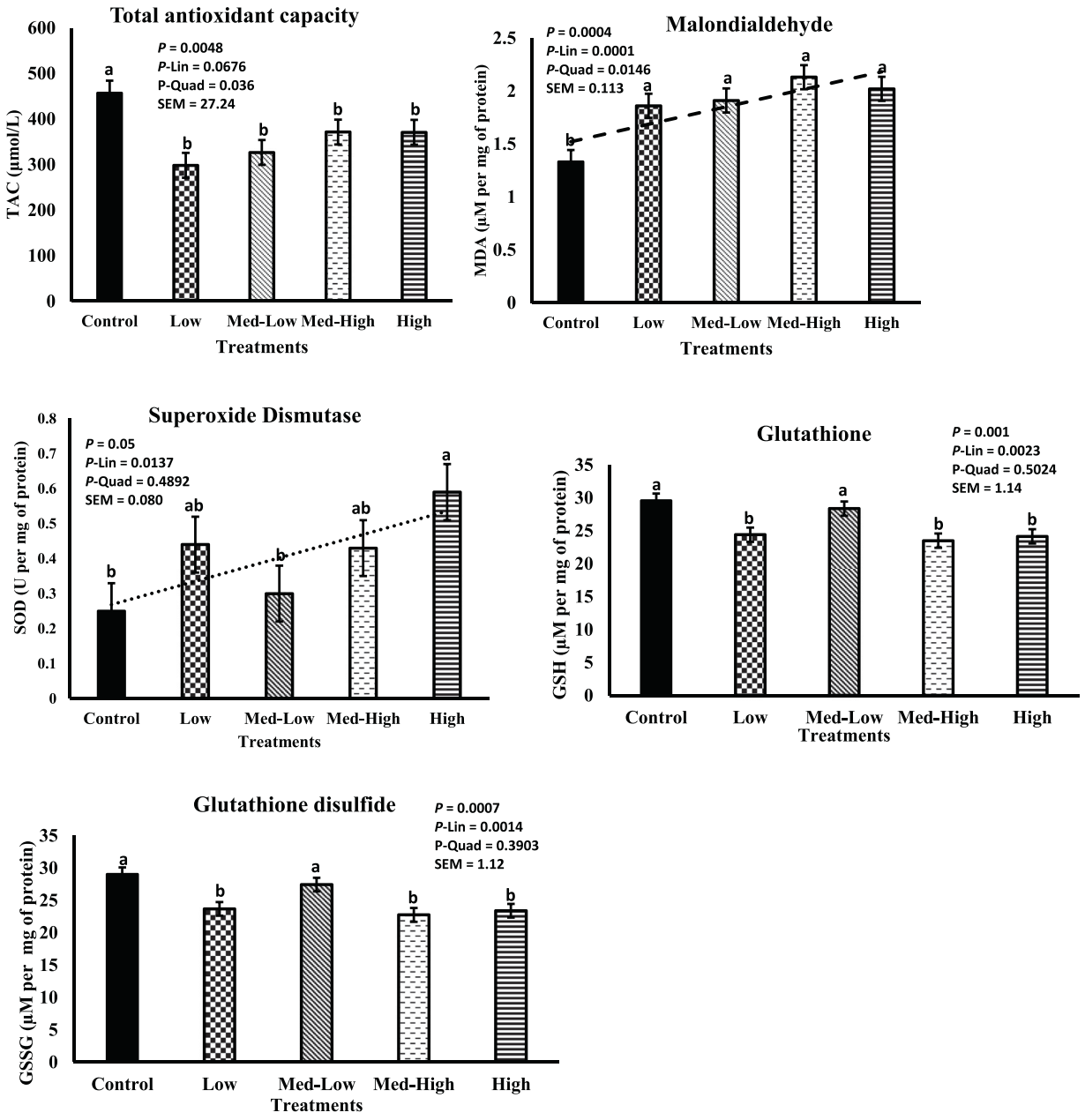

Figure 7. Effect of different dosage of mixed *Eimeria* (*E. maxima, E. acervulina, and E. tenella*) challenge on the oxidative status (TAC, Total antioxidant capacity; MDA, Malondialdehyde; SOD, Superoxide dismutase, GSH,Glutathione; and GSSG, Glutathione disulfide) of Hy-Line W-36 pullets on 6 DPI. The different treatment groups were Control: Nonchallenge control, Low: the low-dose treatment (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina* oocysts per mL), the Med-Low treatment (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina* oocysts per mL), the Med-High treatment (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina* oocysts per mL), and the high-dose treatment (50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* oocysts per mL). Due to high mortality in the high treatment groups, it was terminated from 14 DPI.

upregulate the inflammatory reactions. Intestinal inflammation observed during the coccidiosis is highly correlated with the infiltration of macrophages accompanied by the upregulations of inflammatory cytokines during innate infection stages (Vervelde and Jeurissen, 1995). The upregulation of pro-inflammatory cytokines in the current study was not uncommon and was similar to the previous studies (Hong et al., 2006; Hansen et al., 2021; Gaghan et al., 2022). Hong et al. (2006) proposed that the upregulation of pro-inflammatory cytokines during the coccidiosis may enhance the protective immunity by stimulating the proliferation of chicken T lymphocytes and inflammatory Th1 responses to intracellular pathogenic infection during the adaptive immune response. On the other hand, IL-10 acts as an anti-inflammatory cytokine to inhibit the synthesis of pro-inflammatory cytokines and downregulate the Th1 responses, subsiding the inflammation. The expression of IL-10 was previously reported to be upregulated during *Eimeria* infection similar to our current finding (Rothwell et al., 2004; Hong et al., 2006).

During a host-pathogen interaction, a cellular equilibrium among reactive oxygen species (**ROS**) and host ability to detoxify ROS are disrupted, leading to oxidative stress (Abbas et al., 2013). These ROS have a high affinity for the phospholipid bilayer of the cell membrane

and are produced as a result of host immune response to *Eimeria* infection in chickens. This initiates lipid peroxidation and cytotoxic changes, which in turn damages the intestine and reduces gut integrity (Georgieva et al., 2011; Idris et al., 2019). In the current study, the markers of oxidative stress (TAC, MDA, SOD, GSH, and GSSG) were only significantly different on 6 DPI. Total antioxidant capacity, a measure of the antioxidant abundance status (Bacou et al., 2021), was observed in higher level in the control bird compared to the challenged birds. It indicates that ROS were produced in greater amount in the challenged pullets due to host-*Eimeria* immune response. Malondialdehyde, a marker of radical-induced oxidative stress (Bacou et al., 2021), and SOD, which helps neutralize the reactive oxygen species (Bacou et al., 2021), both increased linearly with the increase in the *Eimeria* dose in the present study. At the same time, GSH and GSSG, capable of preventing damage to important cellular components caused by reactive oxygen species (Bacou et al., 2021), were low in the challenged groups than the control group. These results indicate that the balance between ROS and host ability to neutralize these ROS was disrupted during the *Eimeria* infection due to the host-immune responses, suggesting higher oxidative stress during coccidiosis.

In conclusion, growth performance and body composition responded both linearly and quadratically to the *Eimeria* challenge dosages. Most severe effect on gastrointestinal health was observed on 5 or 6 DPI as suggested by the change in the gastrointestinal permeability, lesion score, and mortality in Hy-Line W-36 pullets. The changes in the nutrient transporter genes, inflammatory cytokines, and oxidative stress during *Eimeria* challenge might be attributed to the host immune response to limit the multiplication of *Eimeria*. This study clearly indicates that *Eimeria* infection can be a threat to gastrointestinal health related issues for pullets and may affect egg production during laying periods. Thus, further studies are necessary to evaluate the impact of *Eimeria* infection in pullets and laying hens on egg production and egg quality during laying periods.

DISCLOSURES

There is no conflict of interest.

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