

# Dietary supplementation with anti-IL-10 antibody during a severe *Eimeria* challenge in broiler chickens

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**ABSTRACT** Attenuation of host IL-10 activity during *Eimeria* infection may elicit a robust Th1 response to eliminate the parasite from the gut epithelium. An experiment was conducted to study the effects of feeding IL-10 neutralizing antibody delivered via a dried egg product (DEP) on growth performance, immune responsiveness, and gut health outcomes during a severe challenge with either *Eimeria acervulina* (study 1) or *Eimeria tenella* (study 2) following FDA CVM #217 protocol to test anticoccidial products. A total of 720 male Ross 308 chicks were used in each study, with 15 replicate cages of 12 birds and the following 4 treatments: sham-inoculated (uninfected) control diet (UCON), *Eimeria*-infected control diet (ICON), and *Eimeria*-infected control diet supplemented with DEP at 2 levels (165 [I-165] or 287 [I-287] U/tonne in study 1 and 143 [I-143] or 287 [I-287] U/tonne in study 2). Individual birds assigned to infected treatment groups received a single oral dose of either 200,000 *E. acervulina* (study 1) or 80,000 *E. tenella* (study 2) oocysts

at 12 d of age (i.e., d post inoculation [DPI] 0), whereas uninfected birds were sham-inoculated with tap water. A one-way ANOVA was performed on outcomes including growth performance, hematology, serum chemistry profiles, immunophenotyping profiles, and intestinal lesion scores. In both studies, DPI 0 to 7 weight gain, feed intake, and feed conversion ratio were worse ( $P < 0.05$ ) in all infected groups compared with the UCON group. Compared with ICON, DEP supplementation elicited no differences on overall growth performance. Histopathology and lesion scores revealed severe damage to the gut epithelium owing to the *Eimeria* challenge, yet DEP supplementation did not improve these outcomes or oocyst shedding, hematological measurements, or serum chemistry. However, DEP supplementation improved ( $P < 0.05$ ) the percentage of circulating CD3<sup>+</sup> cells at 6 DPI in study 2. These results indicate that DEP does not appear to elicit a coccidiostatic effect during a severe infection with *E. acervulina* or *E. tenella*.

**Key words:** IL-10 neutralizing antibody, egg product, *Eimeria*, feed additive, broiler

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## INTRODUCTION

Avian coccidiosis continues to be a problem in the poultry industry and causes poor production performance worldwide. Besides several control measures adopted, complete prevention of the disease seems impossible, especially in intensive poultry rearing. In addition, use of anticoccidial pharmaceuticals has been under scrutiny, and vaccination using live oocysts of *Eimeria* has implications on bird growth and nutrient utilization (Gautier

et al., 2020; Rasheed et al., 2020). However, appropriate immunomodulation and nutritional intervention may be helpful in counteracting the negative effects of *Eimeria* infection on growth (Shanmugasundaram et al., 2013; Morris et al., 2015; Rochell et al., 2017). Host defense against *Eimeria* infection is achieved primarily through cell-mediated immunity, wherein adaptive immune responses play a critical role. Various proinflammatory cytokines (e.g., IL-1 $\beta$ , interferon gamma, and IL-17), chemokines (e.g., IL-8, lymphotactin, migration inhibition factor, and K203), and other immunoregulatory substances (e.g., transforming growth factor-beta 1-4 and granulocyte-macrophage colony-stimulatory factor) along with immune cells (e.g., macrophages, natural killer cells, and T lymphocytes) work together to eliminate the parasite from intestinal epithelial cells. However, IL-10 secreted 4 to 5 d after infection (Hong et al.,

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2006a,b) may have an inhibitory role in parasitic elimination (Shanmugasundaram et al., 2013). During an infection, IL-10 inhibits the transcription factor NF- $\kappa$ B and suppresses the gene transcription of many proinflammatory cytokines including tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , IL-17, interferon gamma, and others (Dokka et al., 2001; Wu et al., 2012). These cytokines secreted predominantly by macrophages, natural killer cells, and Th1 cells serve a critical role in arresting the development of *Eimeria* inside intestinal epithelial cells (Laurent et al., 2001; Park et al., 2007). Collectively, these cytokines stimulate chemokine secretion from fibroblasts and intraepithelial lymphocytes and attract other inflammatory cells including macrophages, neutrophils, and lymphocytes for an amplified immune response. By suppressing the release of proinflammatory cytokines, IL-10 is limiting the host's ability to mount an effective immune response. Therefore, it is possible that *Eimeria*-induced IL-10 production may assist in evasion of the host immune response by *Eimeria* via suppression of interferon gamma-related Th1 responses (Kim et al., 2019).

Passive immunization of hatchlings via immunoglobulins secreted in the egg is a natural method of early protection against diseases in chickens. However, this passive immunization using egg immunoglobulins has been used in preventing/treating gastrointestinal diseases in humans and animals as egg antibodies targeting a particular antigen can be neutralized and removed (Wu et al., 2011; Rahman et al., 2013). For example, antitubulinum neurotoxin egg antibody has been reported to be effective in neutralization of botulinum type A neurotoxin (Trott et al., 2009). Various products targeting a particular antigen or set of antigens have been available on the market such as Globigen22, effective against multiple gastroenteric infections in chickens, and Protimax, targeting F-18 and K-88 strains of *E. coli* infections in pigs and calves (Cook and Trott, 2010). Although purified egg antibodies have limited activity under high temperature and pH, immunoglobulins contained in an egg matrix are somewhat resistant to enzymatic degradation in the gut (Shimizu et al., 1993), and at least 50% of the orally administered immunoglobulins incorporated in egg matrix may be retained throughout the gastrointestinal tract (Warny et al., 1999; Carlander et al., 2000).

In a study by Sand et al. (2016), feeding anti-IL-10 antibody incorporated in whole egg during a mild *Eimeria* infection prevented growth depression in young broilers. However, information is lacking on the efficacy of egg anti-IL-10 antibody during a severe *Eimeria* infection to classify an enriched egg product as an anticoccidial agent. We hypothesized that attenuation of host IL-10 activity by egg anti-IL-10 antibody may elicit an intense proinflammatory reaction and eliminate the parasite during a severe *Eimeria* challenge in broilers. Therefore, the current experiment is designed to evaluate the effects of feeding IL-10 neutralizing antibody in the form of a dried egg product (DEP) on growth performance, immune responsiveness, and gut health outcomes after a severe challenge with either *Eimeria*

*acervulina* (study 1) or *Eimeria tenella* (study 2) following FDA CVM #217 protocol to test anticoccidial products in broilers raised on raised wire flooring in battery cages.

## MATERIALS AND METHODS

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois and of Elanco Animal Health before initiation of the experiment.

### *Birds and Husbandry*

Day-old Ross 308 male broiler chicks not vaccinated against *Eimeria* were obtained from a commercial hatchery and transported to the University of Illinois Poultry Research Unit. Broiler chicks were housed in thermostatically controlled battery cages (model SB5T; Alternative Design Manufacturing, Siloam Springs, AR) with raised wire flooring in an isolated, environmentally controlled room with continuous lighting. A standard corn-soybean meal-based starter diet (Table 1) that meets or exceeds breeder recommendations (Aviagen, 2014) was used to prepare experimental diets, and DEP supplementation (Elanco Animal Health, Greenfield, IN) was included on top of the formulation (i.e., no space reserved in the formulation) as having negligible nutritive contribution to the overall diet. All birds were provided free access to water and the respective experimental diets, which was fed in a single phase in each study.

### *Test Material*

The DEP used herein is a proprietary test article containing IL-10 neutralizing antibody incorporated in whole egg (Elanco Animal Health, Greenfield, IN); the method of manufacture is described by Sand et al. (2016). Antibody titers for the specific lots of DEP used herein were quantified using the enzyme-linked immunosorbent assay (ELISA) before manufacturing of experimental diets; DEP lots were either spray dried (study 1) or granulated (study 2).

### *Eimeria Infection*

Both *E. acervulina* and *E. tenella* used in this study were originally isolated from broiler farms in northwest Arkansas by single oocyst isolation using the agarose gel encapsulation method (Shirley and Harvey, 1996). After isolation, the strains have been continually maintained in the laboratory by periodical propagation of oocysts in 12-day-old broiler chicks. The procedures of propagation and harvesting of oocysts and preparation of oocysts for infection had been described in the study by Shirley (1995). All oocysts used in this study were aged less than 6 wk beyond last propagation.

**Table 1.** Formulation of common basal diet used to prepare experimental diets in studies 1 and 2.

Ingredient, g/kg	Value
Corn	522.0
Soybean meal	400.0
Soy oil	31.3
Salt	4.0
Limestone	13.1
Dicalcium phosphate	18.5
Vitamin premix <sup>1</sup>	2.0
Mineral premix <sup>2</sup>	1.5
L-Lysine HCL	1.2
DL-Methionine	3.5
L-Threonine	0.8
Choline chloride	2.1
Calculated nutrient content, as-is basis	
Crude protein, g/kg	233.7
Calcium, g/kg	10.5
Phosphorus (total), g/kg	7.6
Phosphorus (nonphytate), g/kg	5.0
ME, kcal/kg	3,025
SID <sup>3</sup> amino acids, g/kg	
Arg	14.6
His	5.9
Ile	9.0
Leu	17.6
Lys	12.8
Met	6.8
Met + Cys	9.9
Phe	10.5
Phe + Tyr	16.6
Thr	8.4
Trp	2.6
Val	9.7

<sup>1</sup>Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- $\alpha$ -tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-Ca pantothenate, 10 mg; niacin, 22 mg; menadione sodium bisulfite complex, 2.33 mg.

<sup>2</sup>Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO<sub>4</sub> 7H<sub>2</sub>O; Zn, 75 from ZnO; Cu, 5 from CuSO<sub>4</sub> 5H<sub>2</sub>O; I, 0.75 from ethylenediamine dihydroiodide; Se, 0.1 from Na<sub>2</sub>SeO<sub>3</sub>.

<sup>3</sup>Standardized ileal digestible (SID) AA composition calculated using data acquired from AMINODat 4.0 (Evonik Industries AG, Hanau-Wolfgang, Germany).

## Experimental Design

Two separate studies were conducted to attain the experimental objective, using a total of 720 birds per study. Day-old chicks were weighed, selected, wing banded, and randomly allotted to one of the 4 treatment groups, each with 15 replicates of 12 birds per cage at study initiation, such that the average initial group weights and weight distributions were similar across treatments. The following 4 treatments were used; sham-inoculated (uninfected) control diet (**UCON**), *Eimeria*-infected control diet (**ICON**), and *Eimeria*-infected control diet supplemented with DEP at 2 levels (143 [**I-143**] or 287 [**I-287**] U/tonne in study 1 and 165 [**I-165**] or 287 [**I-287**] U/tonne in study 2). Individual birds assigned to infected groups received a total dose of either 200,000 *E. acervulina* (study 1) or 80,000 *E. tenella* (study 2) oocysts at day 12 (i.e., days post inoculation [**DPI**] 0). The oocyst dosage was suspended in 1 mL of tap water and individually administered by oral gavage, using a repeater pipette fitted with a blunt-ended tip. Noninfected birds were handled similarly and received an oral gavage of tap water at a volume equal to that

of the infected birds. The detailed description of treatment arrangement can be found in [Table 2](#).

## Data and Sample Collection

Group bird and feeder weights were recorded at study day 0 (i.e., DPI 12), 7 (DPI 5), 12 (DPI 0), 19 (DPI 7), and 26 (DPI 14) to calculate body average daily weight gain (**ADG**), feed intake (**FI**), and feed efficiency (feed conversion ratio [**FCR**]) to estimate growth performance. Mortality and culls were monitored daily and used to adjust feed efficiency. A bird was considered study mortality caused by coccidiosis if death/euthanasia occurred after challenge infection and the bird had a necropsy diagnosis of coccidiosis with a gross lesion score >2.5. Oocyst shedding was determined by oocyst counts (i.e., oocysts per gram [**OPG**]) in fecal samples at DPI 0 and 7 from all treatment groups using the McMaster method ([Levine et al., 1960](#)). At DPI 5 and 7, one randomly selected bird in each cage was euthanized with CO<sub>2</sub> to collect blood for hematology and serum chemistry outcomes. In addition, intestinal gross lesion scores were recorded at DPI 7 from either the duodenum (study 1) or ceca (study 2) on a scale of 0 to 4, where 0 means no lesions and 4 means most severe lesions ([Johnson and Reid, 1970](#)).

## Intestinal Histopathology

At DPI 7, samples of the duodenum and upper jejunum (study 1) or ceca (study 2) were collected in 10% neutral buffered formalin from 1 bird per cage and stored at room temperature until analysis. Details of tissue preparation are described by [Oelschlager et al. \(2018\)](#). Tissue sections were examined for histopathological lesions and the physical presence of parasites. A lesion panel developed for commercial poultry production gut assessment was used, and lesions were semi-quantitatively scored for severity as follows: 0, normal; 1, minimal severity; 2, mild severity; 3, moderate; 4, marked; and 5, severe (Veterinary Diagnostic Pathology LLC, Fort Valley, VA). The following lesion scores were recorded: coccidia, villus shortening, crypt hyperplasia (increased depth), lamina propria lymphocytes and plasma cells, bacteria on tips/sides of villi and dysbacteriosis, cystic crypts, intraepithelial leukocytes, and lamina propria heterophils. In addition, the coccidia index was calculated by summing the coccidia scores from each section of the intestine. A cumulative (total) intestinal lesion index was calculated by summing all lesion scores for all intestinal sections, and the total enteritis index was calculated by subtracting the coccidia index from the total lesion index, representing inflammation and repair processes.

## Immunophenotyping

To quantify how dietary treatments affected proportions of T cell subsets after *Eimeria* challenge, whole blood samples were collected from one bird each from

**Table 2.** Details of treatment arrangements in studies 1 and 2.<sup>1</sup>

Treatment	Target DEP supplementation, U/tonne of feed	<i>Eimeria</i> infection
Study 1		
UCON	0	None
ICON	0	<i>E. acervulina</i>
I-143	143	<i>E. acervulina</i>
I-287	287	<i>E. acervulina</i>
Study 2		
UCON	0	None
ICON	0	<i>E. tenella</i>
I-165	165	<i>E. tenella</i>
I-287	287	<i>E. tenella</i>

Abbreviations: DEP, dried egg product; ELISA, enzyme-linked immunosorbent assay; I-143, infected birds receiving 143 U/tonne of DEP; I-165, infected birds receiving 165 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne of DEP; ICON, infected control; UCON, uninfected control.

<sup>1</sup>Both study 1 and 2 were 26-day long, starting at approximately 2 d after hatch, and each treatment included 15 replicate cages of 12 birds (4 treatments  $\times$  15 replicates  $\times$  12 birds). All birds in the infected group received 1 mL of suspension of either 200,000 *E. acervulina* (study 1) or 80,000 *E. tenella* (study 2) sporulated oocysts in water at study day 12. All control birds were sham inoculated with 1 mL of tap water. A common basal starter diet (Table 1) was topped off with the DEP to prepare experimental diets. All diet groups were analyzed for anti-IL-10 activity after diet preparation using ELISA (Sand et al., 2016). The UCON and ICON diet groups were analyzed to contain zero activity in both studies.

a total of 28 cages (i.e., 4 treatments  $\times$  7 randomly selected cages) at DPI 6. The blood samples were used to isolate mononuclear cells and quantify T-cell populations (e.g., total T cells, helper T cells, and cytotoxic T cells) using a flow cytometry technique previously described by Rasheed et al. (2020). In brief, 5 to 8 mL of whole blood collected into EDTA-containing tubes was carefully added to a density gradient (Histopaque; cat. no.: 10771; Sigma-Aldrich, St. Louis, MO) that allowed cells to flow through the gradient. After centrifugation (1,800  $\times$  *g*  $\times$  20 min at 25°C without a brake), mononuclear cells were separated and washed twice with RPMI media (Thermo Fisher Scientific Inc., Waltham, MA), and their concentrations were adjusted to 1  $\times$  10<sup>6</sup> total cells. The cells were then surface stained using the following antibody clones and conjugated fluorochromes: anti-CD3-FITC (clone: CT-3; cat. no.: 8200-02), anti-CD4-PE (clone: CT-4; cat. no.: 8210-09), and anti-CD8 $\alpha$ -APC (clone: CT-8; cat. no.: 8220-11) (SouthernBiotech, Birmingham, AL). After staining, the cells were washed with buffer and permanently fixed with 2% paraformaldehyde for 10 min at room temperature. This was followed by 3 washes, and the cells were kept at 4°C overnight until analysis the following day. The relative percentage of different phenotypes of T cells (i.e., single stain for CD4 or CD8 double-positive T cells) was determined using the multicolor flow cytometry analyzer (BD Biosciences San Jose, CA).

### Statistical Analyses

The experiment was conducted as a completely randomized block design with individual cages as the experimental unit. All data were subjected to a one-way analysis of variance using the MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC). Treatment means

were separated using a Tukey–Kramer adjustment. Outliers were identified (and subsequently removed) as having an absolute studentized residual value of 3 or higher, and significance was accepted with a *P*-value of less than 0.05. For outcomes wherein there were one or more missing values, the highest SEM for any individual was reported as the pooled SEM in the results.

## RESULTS

All diets used in the study were analyzed for anti-IL-10 antibody binding activity using ELISA (Table 3) as described by Sand et al. (2016). An above-target inclusion of anti-IL-10 activity was quantified for all the experimental diets (i.e., 251 and 466 instead of 143 and 287 U/tonne in study 1 and 273 or 597 instead of 165 or 287 U/tonne in study 2).

### Study 1

**Growth Performance and Mortality** Growth performance results are summarized in Figure 1. At DPI 0 to 7 (i.e., study day 12–19), regardless of DEP supplementation, birds in the infected groups exhibited poorer (*P* < 0.04) ADG and FCR than those in the UCON group. Growth performance during DPI 0 to 14 (i.e., study day 19–26) was not different among the *E. acervulina*-infected groups. Overall (study day 0–26), growth performance was not affected by either infection or DEP supplementation. No differences in mortality were measured among treatment groups at any time point (data not shown).

**Oocysts per Gram** Before *E. acervulina* challenge infection at study day 12 (DPI 0), no oocyst counts were reported in any treatment groups, and all birds were free from infection. At DPI 7, there were no differences in OPG among *E. acervulina*-infected groups

**Table 3.** Anti-IL-10 antibody activity of experimental diets used in studies 1 and 2.<sup>1</sup>

Item	Treatment			
	UCON	ICON	I-143 or I-165	I-287
Study 1				
Target activity, U/tonne of feed	0	0	143	287
Measured activity, U/tonne of feed	0	0	273	597
Study 2				
Target activity, U/tonne of feed	0	0	165	287
Measured activity, U/tonne of feed	0	0	251	466

Abbreviations: DEP, dried egg product; ELISA, enzyme-linked immunosorbent assay; I-143, infected birds receiving 143 U/tonne of DEP; I-165, infected birds receiving 165 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne; ICON, infected control; UCON, uninfected control.

<sup>1</sup>No anti-IL-10 activity was quantified in the unsupplemented control diet group from either study. Diet analysis was performed using a validated ELISA procedure (Sand et al., 2016).

(Figure 2). No oocysts were found in the UCON group, confirming that these birds remained free from inadvertent infection.

**Hematology** At DPI 5, I-143 birds exhibited higher ( $P < 0.011$ ) heterophil and lower ( $P < 0.001$ ) lymphocyte percentages than those in all other infected groups (Table 4). All the infected groups showed a lower ( $P < 0.01$ ) total plasma protein concentration than the UCON group. In addition, monocyte percentages were lowered ( $P = 0.001$ ) in the I-287 group, and basophil levels were elevated ( $P = 0.007$ ) in the I-143 group. No other hematology parameters were affected at DPI 5. At DPI 7, hematocrit and eosinophil percentages were lowered ( $P < 0.009$ ) and monocyte levels were elevated ( $P = 0.007$ ) in DEP-supplemented groups compared with the UCON or ICON group. Basophil percentages were elevated ( $P = 0.03$ ) only in I-143 birds.

**Serum Chemistry** Liver enzymes aspartate aminotransferase, creatine phosphokinase, glutamate dehydrogenase and albumin concentrations were not affected by *E. acervulina* infection or DEP supplementation at any time point (data not shown). At DPI 5, although the calcium level was lowered ( $P < 0.001$ ) in the I-143 group only, the blood glucose level was elevated ( $P = 0.004$ ) both in the I-143 and I-287 groups compared with the ICON group (Table 4). No serum chemistry parameters were affected at DPI 7.

**Histopathological Lesion Scoring** As shown in Table 5, enteritis scores in DEP-supplemented groups were not different compared with the those in ICON group. Birds in the I-287 group exhibited a lower ( $P < 0.009$ ) bacterial dysbiosis and heterophil score and a higher ( $P = 0.093$ ) villous atrophy score than those in the ICON group.

**Immunophenotyping** T-cell populations in blood (i.e., helper T cells, cytotoxic T cells, and memory T cells) measured at DPI 6 were not affected by either *E. acervulina* infection or supplementation with DEP (data not shown).

## Study 2

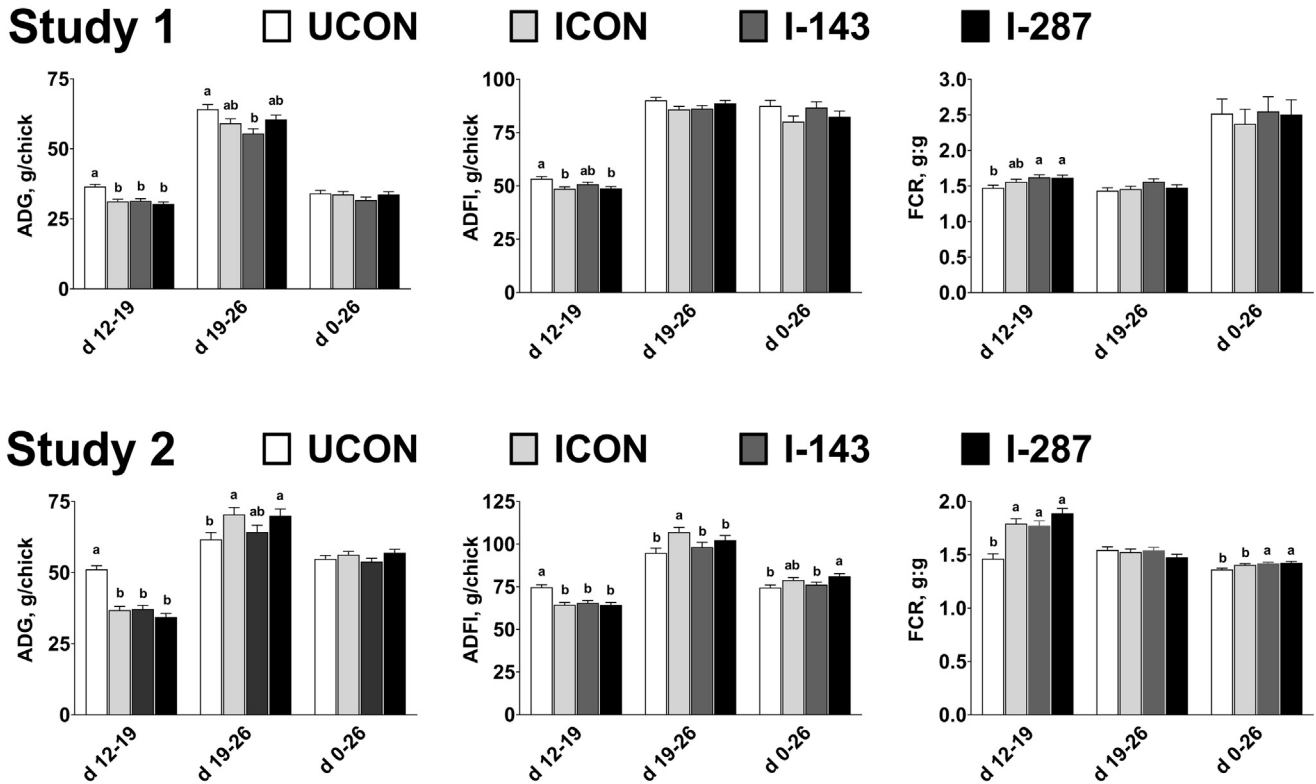
**Growth Performance and Mortality** At DPI 0 to 7 (i.e., study day 12–19), all infected groups showed poorer

( $P < 0.001$ ) ADG, FI, and FCR than the UCON group, and DEP supplementation did not improve growth performance parameters compared with ICON (Figure 1). At DPI 7 to 14 (i.e., study day 19–26), although ICON and I-287 birds exhibited increased ( $P = 0.004$ ) ADG, ICON birds had higher ADFI ( $P = 0.004$ ) than UCON birds. However, FCR at was not at all different among the groups. Overall (i.e., study day 0–26), *E. tenella* challenge worsened FCR compared with UCON. In addition, there were no differences in ADG among the *E. tenella*-infected groups. No difference in mortality was observed among the treatment groups at DPI 12 to 5 (i.e., study day 0–7), DPI 5 to 0 (i.e., study day 7–12), or DPI 7 to 14 (i.e., study day 19–26). However, immediately after the challenge (DPI 0–7), mortality was higher ( $P < 0.007$ ) in all the infected groups than in the UCON group. Mortality was not different among the *E. tenella*-infected groups (data not shown).

**Oocysts per Gram** No oocysts were found in UCON birds. A relatively low number of oocysts were reported from 2 cages of ICON before infection at DPI 0 (data not shown), which was postulated to be due to a cross contamination of excreta pans that had not been fully disinfected from previous studies, and not infection of the birds in study. Because excreta pans were physically located in such a way that birds had no access to excreta, it was unlikely that ICON birds were infected before the day of inoculation. At DPI 7, I-287 birds had a higher ( $P < 0.022$ ) OPG than other infected groups (Figure 2).

**Hematology** Hematology parameters are shown in Table 4. At DPI 5, hematocrit was lower ( $P = 0.005$ ) in all the infected groups except the I-287 group, and basophil percentage and plasma total protein levels were reduced ( $P < 0.023$ ) in I-165 compared with UCON; no other parameters were affected at DPI 5. At DPI 7, I-287 birds exhibited a lower ( $P = 0.009$ ) hematocrit than I-165 birds. In addition, all infected groups had an elevated ( $P = 0.007$ ) monocyte percentage compared with the ICON group; no other hematological parameters were affected at this time point.

**Serum Chemistry** At DPI 5, glucose concentration was elevated ( $P = 0.001$ ) in I-287 birds compared with ICON birds (Table 4). In addition, the albumin level



**Figure 1.** Growth performance parameters of birds receiving the dried egg product at 2 levels: 143 or 287 U/tonne in study 1 and infected with either *E. acervulina* (200,000 oocysts per bird; study 1) or *E. tenella* (80,000 oocysts per bird; study 2) at study day 12. The study periods represented are day 0 to 7, DPI 12 to 5; day 7 to 12, DPI 5 to 0; day 12 to 19, DPI 0 to 7; and day 19 to 26, DPI 7 to 14. <sup>a,b</sup>Means lacking a common superscript letter within a group of bars differ ( $P < 0.05$ ). Least square means derived from samples from 15 replicate battery cages ( $n = 15$ ) at each independent time point. Abbreviations: ADG, average daily weight gain; DEP, dried egg product; DPI, day post infection; FCR, feed conversion ratio; I-143, infected birds receiving 143 U/tonne of DEP; I-165, infected birds receiving 165 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne of DEP; ICON, infected control; UCON, uninfected control.

was elevated ( $P < 0.001$ ) in all infected groups compared with the UCON group. Serum chemistry was not affected by either infection or DEP supplementation at DPI 7.

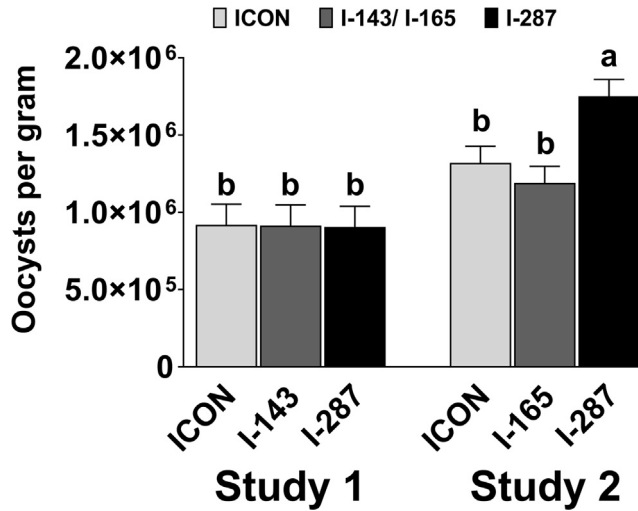
**Histopathological Lesion Scoring** The overall coccidia index scores were reported normal (severity score = 0) for UCON birds and severe (severity score = 5) for all infected groups. Therefore, no statistical comparison to UCON was conducted, given the lack of variation for this group. However, higher ( $P = 0.005$ ) total enteritis scores and total lesion scores were observed in DEP-supplemented groups compared with the ICON group. In addition, I-287 birds exhibited severe scores ( $P = 0.005$ ) of cystic crypts and lymphoid hyperplasia scores compared with other infected groups, and a higher ( $P = 0.02$ ) fibroblast hyperplasia score was observed in both the DEP-supplemented groups than in the ICON group (Table 5).

**Immunophenotyping** Immunophenotyping was performed at DPI 6, and results are summarized in Table 6. Birds in the I-287 group exhibited a higher ( $P = 0.002$ ) percentage of CD3<sup>+</sup> cells than both UCON and I-165 birds. No other T-cell subsets including helper (CD3+CD4+CD8<sup>-</sup>), cytotoxic (CD3+CD4<sup>-</sup>CD8<sup>+</sup>), and memory (CD3+CD4+CD8<sup>+</sup>) T cells were affected by experimental treatments.

## DISCUSSION

Supplementation of oral egg antibodies to neutralize host proteins that benefit the host's immune system has already been established (reviewed by Cook and Trott, 2010; Rahman et al., 2013). Egg-derived anti-IL-10 antibodies were shown to be beneficial during a mild *Eimeria* infection (Sand et al., 2016); in the current experiment, we tested its efficacy during a severe and short-term infection. An above-target inclusion of anti-IL-10 activity was quantified in the diet, and this higher level should have provided adequate antibody activity at the gut epithelium as approximately 50% of the antibody is expected to survive the digestive process (Warny et al., 1999; Carlander et al., 2000). However, in the present study, we did not quantify anti-IL-10 activity of luminal contents throughout the gastrointestinal tract, so we cannot definitively confirm this point.

The challenge used in the current experiment elicited a 14 to 17% (study 1) or 27 to 32% (study 2) growth depression in the infected groups at 7 DPI, indicating that the infection was successful and adequately severe when compared with the UCON group. By inducing a severe infection, our aim was to assess the ability of the DEP to be considered as an anticoccidial agent. Growth performance and oocyst output measurements



**Figure 2.** Oocysts per gram of feces 7 d post infection (7 DPI) in birds receiving the dried egg product at 2 levels: 143 or 287 U/tonne in study 1 and 165 or 287 U/tonne in study 2 and infected with either *E. acervulina* (200,000 oocysts per bird; study 1 and study 2) at study day 12. <sup>a, b</sup>Means lacking a common superscript letter within a group of bars differ ( $P < 0.05$ ). Least squares means derived from samples from 15 replicate battery cages ( $n = 15$ ) at each independent time point. No oocysts were detected from the UCON group and hence not included in statistical analysis. Abbreviations: DEP, dried egg product; I-143, infected birds receiving 143 U/tonne of DEP; I-165, infected birds receiving 165 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne of DEP; ICON, infected control; UCON, uninfected control.

are considered as primary outcomes in *Eimeria* infection studies in chickens (Holdsworth et al., 2004). *Eimeria* causes poor nutrient absorption from the gut

leading to poor growth, and hence, growth performance measurements are critical (Chapman, 1998; Lee et al., 2009a,b). Similarly, the amount of *Eimeria* oocyst shedding via feces represents direct evidence of a host’s immunity to arrest parasitic development in the gut epithelium (Chapman, 1999; Parmentier et al., 2001; Lee et al., 2013). When birds fed with anti-IL-10 antibody were challenged with a 10× dose of *Eimeria* vaccine, Sand et al. (2016) observed improvements in growth performance and oocyst output. In addition, anti-IL-10 antibody is hypothesized to prevent the completion of *Eimeria* life cycle owing to a boosted intestinal inflammatory response. However, it did not interfere with the development of immunity on early vaccination (i.e., vaccination with a 1× dose of *Eimeria* vaccine at day 3), which itself is an immune challenge (Sand et al., 2016). These results suggest that anti-IL-10 antibody could be beneficial during a severe challenge infection, allowing the development of immunity at the same time as if birds had been vaccinated. In the present study, although we did not vaccinate the birds, anti-IL-10 antibody at the given dose may not improve growth performance and oocyst shedding during a severe and short-term *Eimeria* infection. Reduced feed utilization either without or with a reduction in FI is considered a primary reason for losses in weight gain during *Eimeria* infection (Kipper et al., 2013). Overall, day 0 to 26 ADG was not affected owing to either *Eimeria* infection or DEP supplementation, suggesting that birds showed signs of recovery from the infection, and DEP supplementation did not negatively affect growth.

**Table 4.** Hematological and clinical chemistry outcomes of birds receiving the dried egg product containing anti-IL-10 antibody via feed during a challenge infection with *Eimeria*.<sup>1</sup>

Item	Outcome	Treatment				Pooled SEM	Model, <i>P</i> -value
		UCON	ICON	I-143 or I-165	I-287		
<b>Study 1</b>							
DPI 5	Heterophils, %	29.27 <sup>b</sup>	18.93 <sup>c</sup>	38.67 <sup>a</sup>	16.73 <sup>c</sup>	3.053	<0.001
	Monocytes, %	2.60 <sup>b</sup>	6.93 <sup>a</sup>	7.86 <sup>a</sup>	3.80 <sup>b</sup>	0.868	0.001
	Lymphocytes, %	64.73 <sup>a</sup>	71.20 <sup>a</sup>	46.80 <sup>b</sup>	76.46 <sup>a</sup>	3.426	<0.001
	Basophils, %	2.93 <sup>b</sup>	2.40 <sup>b</sup>	5.20 <sup>a</sup>	2.53 <sup>b</sup>	0.624	0.007
	Total protein, g/dL	2.83 <sup>a</sup>	2.56 <sup>b</sup>	2.52 <sup>b</sup>	2.57 <sup>b</sup>	0.044	<0.001
	Glucose, mg/dL	300.27 <sup>b</sup>	355.21 <sup>b</sup>	404.87 <sup>a</sup>	376.13 <sup>a</sup>	20.210	0.004
	Calcium, mg/dL	8.88 <sup>a</sup>	8.52 <sup>a</sup>	7.31 <sup>b</sup>	7.71 <sup>a</sup>	0.338	<0.001
	Hematocrit, %	30.80 <sup>a</sup>	28.87 <sup>a,b</sup>	28.27 <sup>b</sup>	28.07 <sup>b</sup>	0.609	0.009
	Monocytes, %	3.13 <sup>b</sup>	6.53 <sup>b</sup>	7.47 <sup>a</sup>	7.47 <sup>a</sup>	0.980	0.007
	Basophils, %	3.07 <sup>b</sup>	4.73 <sup>b</sup>	6.07 <sup>a</sup>	5.20 <sup>a,b</sup>	0.717	0.030
Eosinophils, %	1.13 <sup>a,b</sup>	1.47 <sup>a</sup>	0.27 <sup>c</sup>	0.40 <sup>b,c</sup>	0.276	0.007	
<b>Study 2</b>							
DPI 5	Hematocrit, %	25.63 <sup>a</sup>	16.00 <sup>b</sup>	14.86 <sup>b</sup>	18.71 <sup>a,b</sup>	2.631	0.005
	Total protein, g/dL	2.85 <sup>a</sup>	2.67 <sup>a,b</sup>	2.53 <sup>b</sup>	2.54 <sup>a,b</sup>	0.106	0.023
	Basophils, %	4.53 <sup>a</sup>	2.40 <sup>a</sup>	1.50 <sup>b</sup>	1.71 <sup>a,b</sup>	0.879	0.005
	Albumin, g/dL	0.94 <sup>b</sup>	1.39 <sup>a</sup>	1.29 <sup>a</sup>	1.34 <sup>a</sup>	0.063	<0.001
	Glucose, mg/dL	316.53 <sup>b</sup>	312.07 <sup>b</sup>	275.46 <sup>b</sup>	392.33 <sup>a</sup>	21.107	0.001
DPI 7	Hematocrit, %	25.73 <sup>a,b</sup>	28.00 <sup>a</sup>	26.86 <sup>a,b</sup>	24.83 <sup>b</sup>	0.749	0.009
	Monocytes, %	1.67 <sup>b</sup>	5.93 <sup>a</sup>	5.85 <sup>a</sup>	8.00 <sup>a</sup>	1.095	0.007

<sup>a-c</sup>Means lacking a common superscript letter within a row differ ( $P < 0.05$ ).

Abbreviations: DEP, dried egg product; DPI, d post infection; I-143, infected birds receiving 143 U/tonne of DEP; I-165, infected birds receiving 165 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne of DEP; ICON, infected control; UCON, uninfected control.

<sup>1</sup>Only significant results are shown. The dried egg product was supplemented at a dose of 143 or 287 (study 1) and 165 or 287 U/tonne (study 2). All birds in the infected groups received an oral inoculum containing either 200,000 *E. acervulina* (study 1) or 80,000 *E. tenella* (study 2) sporulated oocysts at study day 12. Least square means derived from samples derived from 1 bird in each of the 15 replicate battery cages ( $n = 15$ ) at each independent collection time point.

**Table 5.** Summary of histopathological lesion scores of individual birds receiving the dried egg product via feed during a challenge infection with either *E. acervulina* (study 1) or *E. tenella* (study 2) at day 12.<sup>1</sup>

Item	Treatment			Pooled SEM	Model, <i>P</i> -value
	ICON	I-143 or I-165	I-287		
Study 1					
Villous atrophy	2.14 <sup>b</sup>	2.25 <sup>a</sup>	2.50 <sup>a</sup>	0.093	0.025
Heterophils	1.44 <sup>a</sup>	1.82 <sup>a</sup>	0.82 <sup>b</sup>	0.174	0.009
Bacterial dysbiosis	1.61 <sup>a</sup>	1.68 <sup>a</sup>	0.93 <sup>b</sup>	0.162	0.004
Enteritis score	16.24 <sup>a,b</sup>	16.75 <sup>a</sup>	15.00 <sup>b</sup>	0.474	0.036
Study 2					
Lymphoid hyperplasia	2.60 <sup>b</sup>	2.51 <sup>b</sup>	3.18 <sup>a</sup>	0.182	0.014
Fibroblast hyperplasia	3.20 <sup>b</sup>	4.05 <sup>a</sup>	4.35 <sup>a</sup>	0.225	0.003
Cystic crypts	1.00 <sup>b</sup>	2.29 <sup>a</sup>	2.44 <sup>a</sup>	0.243	0.002
Enteritis score	12.87 <sup>b</sup>	15.29 <sup>a,b</sup>	17.38 <sup>a</sup>	0.967	0.005
Total lesion score	17.87 <sup>b</sup>	20.29 <sup>a,b</sup>	22.38 <sup>a</sup>	0.967	0.005

<sup>a,b</sup>Means lacking a common superscript letter within a row differ ( $P < 0.05$ ).

Abbreviations: DEP, dried egg product; I-143, infected birds receiving 143 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne of DEP; ICON, infected control; UCON, uninfected control.

<sup>1</sup>Only significant results are shown. The UCON group was not included in the statistical analysis owing to lack of variation in the data. All birds in the infected groups received an oral inoculum containing 200,000 sporulated oocysts of *E. acervulina*.

Generally, severity of *Eimeria* infection is reflected in bird mortality. However, this is not true for *E. acervulina* as it has a low mortality rate even at high infection doses (Chapman, 1998). In the present study, all groups infected with *E. tenella*, but not *E. acervulina*, had a higher mortality rate than the UCON group. Mortality and decreases in growth performance during DPI 0 to 7 is closely associated with pathological damage to the gut wall (Giannenas et al., 2014). In the current experiment, the coccidia index (indicating the amount of coccidia infection), enteritis score, total lesion score, and villous atrophy score (indicating severity of inflammatory process) were higher in all infected treatments compared with UCON treatment, suggesting severe inflammation due to coccidia infection, and DEP did not improve these outcomes. However, birds receiving 287 U/tonne of DEP in study 1 had lower bacterial dysbiosis, heterophil, and cystic crypts scores after an *E. acervulina* challenge, indicating a beneficial effect of the DEP in improving gut health. A lower bacterial dysbiosis score indicates better gut microflora balance and

improved gut health (Bailey, 2010; Le Roy et al., 2019), and a lower heterophil score is associated with a lower bacterial dysbiosis score (Naghizadeh et al., 2019). Cystic crypts are generally seen during viral infections (Frazier and Reece, 1990; Davis et al., 2013), and DEP supplementation reduced cystic crypts. During an *E. tenella* challenge in study 2, higher fibroblast hyperplasia, lymphoid hyperplasia, and heterophil scores in the DEP-supplemented group indicate severe inflammation and poor gut health. Collectively, our results suggest that the dietary DEP could be beneficial in promoting gut health during a severe *E. acervulina* infection, but possibly not during a severe *E. tenella* infection. This difference could be due to the variation in pathology of these species as *E. tenella* is more pathogenic than *E. acervulina* (Johnson and Reid, 1970).

Increases in intestinal T-cell subsets (CD3+, CD4+, and CD8+) are important for host resistance against *Eimeria* infection (Hong et al., 2006a,b; Swinkels et al., 2007), and increased CD3+ T-cell populations aid in a better immune response (Song et al., 2010). Although

**Table 6.** Peripheral blood T-cell immunophenotypes of birds 6 d post infection with *E. tenella* and supplemented with the dried egg product containing anti-IL-10 antibody via feed (study 2).<sup>1</sup>

Outcome <sup>2</sup>	Treatment				Pooled SEM	Model, <i>P</i> -value
	UCON	ICON	I-165	I-287		
Total T cells, % (CD3 <sup>+</sup> )	6.01 <sup>b</sup>	6.68 <sup>b</sup>	13.58 <sup>b</sup>	26.67 <sup>a</sup>	4.278	0.002
Helper T cell, % (CD3 <sup>+</sup> CD4 <sup>+</sup> )	2.19	1.93	1.89	4.37	1.223	0.304
Cytotoxic T cell, % (CD3 <sup>+</sup> CD8 <sup>+</sup> )	0.47	1.29	1.32	1.74	0.467	0.175
Memory T cell, % (CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>+</sup> )	0.03	0.04	0.08	0.05	0.024	0.355

<sup>a, b</sup>Means lacking a common superscript letter within a row differ ( $P < 0.05$ ).

Abbreviations: DEP, dried egg product; I-165, infected birds receiving 165 U/tonne of DEP; I-287, infected birds receiving 287 U/tonne of DEP; ICON, infected control; UCON, uninfected control.

<sup>1</sup>The DEP was supplemented at a dose of 165 or 287 U/tonne. All birds in the infected groups received an oral inoculum containing 80,000 sporulated oocysts of *E. tenella*. Least square means derived from samples derived from 1 bird in each of 7 replicate battery cages ( $n = 7$ ) at each independent collection time point.

<sup>2</sup>Total T cells were expressed as a percentage of total cells isolated by extraction of peripheral blood mononuclear cell extraction and expressing the CD3 cell surface marker. Helper, cytotoxic, and memory T-cell populations are expressed as a percentage of total CD3<sup>+</sup> T cells.



not measured at the site of infection or in lymphoid organs, DEP supplementation increased the percentage of total CD3+ cells (T cells exhibiting CD3 surface markers) in peripheral circulation after *E. tenella* infection in study 2. However, peripheral T-cell subsets were not affected during *E. acervulina* infection. Immunophenotyping of cecal tonsils may have provided a better understanding of difference in effects between *E. acervulina* and *E. tenella* infections as the T-cell response at the site of infection is robust.

Hematology and serum chemistry profiles were measured to study the effect of the DEP and *Eimeria* infection on systemic circulation. Because host response to *Eimeria* infection is localized to the site of infection (i.e., intestinal wall), only a few studies have investigated the effect of *Eimeria* infection on hematological and serum chemistry parameters. Loss of blood at the site of *Eimeria* infection can cause lower hematocrit and plasma protein levels with an increase in lymphocyte and heterophil counts (Adamu et al., 2013; Akhtar et al., 2015). In agreement to this observation, the current results confirmed lower total plasma protein (DPI 5 in study 1) and hematocrit (DPI 7 in study 1 and DPI 5 in study 2) levels and higher heterophil percentages (DPI 5 in study 1) in at least one of the infected groups, regardless of DEP supplementation. However, lymphocyte percentages were either elevated (ICON and I-287 at DPI 5) in study 1 or unaffected in study 2. Unlike reduced serum enzyme concentrations expressed by Adamu et al. (2013), no serum enzymes were affected in the present study either owing to *Eimeria* infection or owing to DEP supplementation. Serum enzymes are markers of organ damage (e.g., aspartate aminotransferase and ALT indicate liver damage, and creatine phosphokinase indicates muscle damage), and *Eimeria* is not reported to cause liver or muscle damage in chickens. As the infection is localized only to the intestinal epithelium, we conclude that *Eimeria* infection may not have pathological effects on liver and muscle tissues, and no effects of DEP supplementation were observed for these outcomes either, suggesting it was safe. Other parameters including serum albumin, Ca, P, and blood glucose levels showed no consistent changes due to infection or DEP supplementation between studies 1 and 2. In general, serum chemistry and blood parameters are not considered strong indicators of health and immunity during *Eimeria* infection in chickens. The current results are not helpful in drawing a meaningful conclusion about the effects of treatments on serum and hematology parameters.

In conclusion, the current results obtained following FDA CVM #217 protocol to test anticoccidial products under severe *E. acervulina* or *E. tenella* challenge indicate that regardless of dosage, the DEP containing anti-IL-10 antibody should not be considered or used as an anticoccidial product. However, a higher percentage of CD3+ cells in groups supplemented with DEP at a dose of 287 U/tonne at DPI 6 with *E. tenella* (study 2) suggests a beneficial cellular immune response. Although not measured in this study, a cytokine analysis

of intestinal epithelium samples may have been beneficial in precisely understanding the effect of DEP supplementation on improved CD3+ cells.

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