

Correlation between Intestinal Health and Coccidiosis Prevalence in Broilers during Different Seasons of the Year in Brazil from 2012 to 2018

Fabio Luis Gazoni^{1*}, Gabriela Miotto Galli², Marcel Manente Boiogo³, Lenita Moura Stefani³, Aline Zampar³, Marco A. Juárez-Estrada⁴, Guillermo Tellez-Isaias⁵, Aleksandro S. da Silva^{3*}

¹Animal Health and Reproduction, Veterinary Medicine Graduate Program, Federal University of Santa Maria (UFSM), Santa Maria, Brazil

²Animal Science Graduate Program, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

³Animal Science Graduate Program, Santa Catarina State University (UDESC), Chapecó, Brazil

⁴Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de Mexico, Ciudad Universitaria, Ciudad de Mexico, Mexico

⁵Department of Poultry Science, Division of Agriculture, University of Arkansas, Fayetteville, AR, USA

Email: *gazonivet@gmail.com, *aleksandro.silva@udesc.br

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Abstract

Coccidiosis is a disease caused by intracellular protozoan parasites, specifically belonging to the genus *Eimeria*. These parasites target the gastrointestinal tract in different types of hosts, causing sores in the intestinal lining. The presence of these lesions reduces the animal's ability to digest and absorb nutrients, significantly impacting their overall performance. The current study aimed to explore the potential correlation between seasonal variations and the incidence of *Eimeria* spp.-induced lesions in broiler chickens' gastrointestinal tracts in Brazil from 2012 to 2018. A total of 8,607 broiler chickens, aged 14 to 42 days, were sampled from 103 poultry integrated companies in Brazil to conduct intestinal health examinations. The sampling process involved selecting 3 to 6 chickens from each poultry house for examination. The assessment included various abnormalities such as shedding of intestinal cells and excessive fluid and mucus presence, thickening and tension of the intestines, food movement, roughened mucosal surface resembling a Turkish towel in the small intestine, tissue death, duodenal inflammation, intestinal inflammation, gizzard erosion, presence of worms and bedding material, and ingestion of mealworms. During the seasons, winter exhibited the highest average occurrence of *Eimeria maxima* microorganisms at 52.83%, with *E. acervulina* following closely at 26.42% in second place. In spring, *E. maxima* had an occurrence of 11.31%,

while in fall, *E. tenella* had the lowest occurrence at 6.74%. When analyzing the seasonal occurrence of *Eimeria*, it was observed that *E. maxima* micro was more common during winter compared to summer ($P = 0.0491$). However, no discernible variation was observed in the occurrence of the remaining species across different seasons. Research findings suggest that subclinical coccidiosis is most prevalent during the winter season in Brazil. Likewise, clinical disease caused by *E. acervulina* is also prevalent during this time. In contrast, *E. maxima* is more likely to cause clinical disease in the spring, whereas *E. tenella* is more commonly associated with clinical disease in the fall. Lesions induced by *Eimeria* spp. are associated with factors influencing the overall health of broiler intestines. These findings allow for the utilization of seasonal metrics in disease management, thereby reducing economic losses associated with the condition.

Keywords

Eimeria, Epidemiology, Intestinal Pathology, Broiler Chickens, Brazil

1. Introduction

In 2020, Brazil held the top position as the largest exporter of chicken meat, with a total export volume of 13.845 million tons. Furthermore, the average *per capita* consumption of chicken meat in Brazil was reported to be 45.27 kg per person. To maintain optimal productivity in this setting, it is crucial to efficiently manage infections that pose a threat to the poultry industry, such as coccidiosis. This parasitic infection, caused by protozoa of the genus *Eimeria* spp., leads to significant losses due to illness and mortality. In 2016, it was estimated that this illness incurred annual costs worldwide ranging from \$7 to \$13 billion [1].

The *Eimeria* species commonly found in poultry birds comprise *Eimeria acervulina*, *Eimeria maxima*, *Eimeria brunetti*, *Eimeria mitis*, *Eimeria praecox*, *Eimeria necatrix*, and *Eimeria tenella*. This pathogen undergoes intracellular development in specific regions of birds' gastrointestinal tract, leading to reduced weight gain and increased feed conversion. This is due to severe damage to the intestines, resulting in increased intestinal permeability. Additionally, it induces excess mucus and fluid, intestinal desquamation, and erosion of the gizzard. Furthermore, coccidiosis disrupts intestinal energy metabolism by affecting ATP production and consumption, leading to inflammation and oxidative stress [2]. Consequently, when intestinal balance is disrupted, birds become more prone to other illnesses like *Clostridium perfringens*. This impacts both the health and productivity of the birds [3].

Due to Brazil's vast geographical expanse and varied climates, it is imperative to fully comprehend how animal intestinal health relates to coccidiosis throughout different seasons. Understanding this relationship is crucial for developing diverse prevention strategies aimed at reducing the incidence of coccidiosis and

mitigating its economic impact on the poultry industry.

However, there is limited and contradictory evidence regarding the occurrence of coccidiosis across various seasons. Razmi and Kalideri (2000) [4] noted a higher incidence of clinical and/or subclinical coccidiosis during the spring and winter seasons. In contrast, Ahad *et al.* (2014) [5] reported a higher incidence during the autumn and summer seasons. This study aimed to determine if there is a connection between seasonal variations and the presence of specific lesions induced by *Eimeria* spp. in the gastrointestinal tract of broilers in Brazil from 2012 to 2018.

2. Materials and Methods

2.1. Animals and Data Set

A study was undertaken to assess the intestinal health of broiler chickens across 103 poultry companies located in different states of Brazil from 2012 to 2018. The study involved a total of 8607 chickens from the Cobb and Aviagen lines, aged between 14 and 42 days. It is important to note that this study was purely observational. Consequently, the feed given to the birds originated from their respective integrated companies, with no involvement or direction from the evaluator concerning the content or utilization of growth promoters or anticoccidial drugs. Additionally, the evaluated broilers did not undergo any coccidiosis immunization programs. The data from all four seasons - spring, summer, autumn, and winter - was analyzed to determine the correlation between the presence of intestinal lesions and the different seasons.

2.2. Sampling

The assessment was conducted on a random selection of 3 to 6 avian specimens from each poultry barn, specifically at three distinct locations within the chicken building (entrance, middle, and exit). Euthanasia was conducted via cervical dislocation, in accordance with the animal welfare and euthanasia guidelines outlined by Brasil/MCTI in 2013 [6]. The macroscopic evaluation involved examining cellular desquamation, excessive fluid and mucus, ingestion of bedding and mealworms, alterations in intestinal thickness and tone, food passage, presence of worms (*Raillietina* spp.), duodenitis, enteritis, necrotic enteritis, and erosion of the gizzard [7]. **Figure 1** depicts the occurrence of lesions induced by *E. acervulina*, *E. maxima*, and *E. tenella* in the gastrointestinal tract. Lesions were categorized based on their severity using the Johnson and Reid (1970) [8] methodology, with a score of 0 representing no injury and a score of 4 indicating severe damage.

Furthermore, the microscopic examination (at a magnification of 100×) evaluated the existence of *E. maxima* oocysts in the intestinal mucosa surrounding the Meckel's diverticulum. This assessment was conducted at five specific locations on the slide, which included the four corners and the center. The microscopic scores were assessed on a scale of zero to four, where zero indicated the

absence of oocysts, one indicated 1 to 10 oocysts, two indicated 11 to 20 oocysts, three indicated 21 to 40 oocysts, and four indicated more than 41 oocysts [9], as depicted in **Figure 2**.

The species *E. acervulina* ($18.3 \times 14.6 \mu\text{m}$), *E. maxima* ($30.5 \times 20.7 \mu\text{m}$), and *E. tenella* ($22 \times 19 \mu\text{m}$) were assessed according to a previous study conducted by [7]. The results can be seen in **Figure 3**.

2.3. Statistical Analysis

The normality test (Shapiro-Wilk) was applied to all variables. Subsequently, the data underwent analysis of variance (ANOVA) using SAS 9.3 software, and correlation tests between lesions and necropsy findings during the four seasons of the year. A difference between treatments was considered significant when $P = 0.05$.

3. Results

Characteristics such as cell desquamation, excessive fluid and mucus, ingestion of bedding and mealworms, intestinal thickness, food passage, intestinal tone, Turkish towel appearance, presence of worms (*Raillietina* spp.), duodenitis, enteritis, necrotic enteritis, and gizzard erosion were evaluated as described by [7]. **Table 1** displays the relationships among intestinal changes during the several seasons of the year, namely Summer (S), Autumn (A), Winter (W), and Spring



Figure 1. Presence of lesions caused by *E. acervulina*, *E. maxima*, and *E. tenella*.

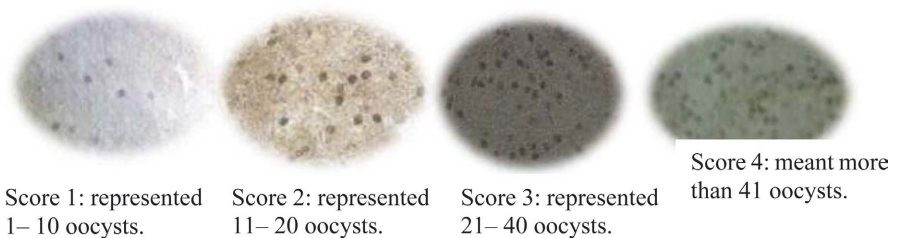


Figure 2. Four scoring categories were utilized for classifying *Eimeria maxima* micro assay.

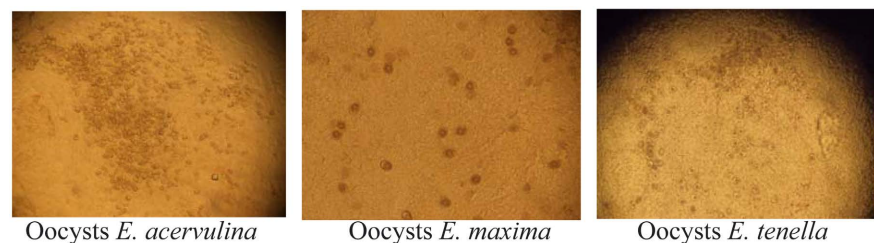


Figure 3. Detection of oocysts for *E. acervulina*, *E. maxima*, and *E. tenella*.

Table 1. Correlation between broiler’s intestinal alterations traits and annual seasons.

	Excess fluid	Excess mucus	Litter intake	Mealworm intake	Thick intestine	Thin intestine	Passage feed	Altered intestinal tone	Turkish towel appearance	Worms	Necrotic enteritis	Duodenitis	Enteritis	<i>Eimeria acervulina</i>	<i>Eimeria maxima</i>	<i>Eimeria tenella</i>	<i>Eimeria maxima</i> micro	Gizzard erosion
Cell desquamation	S-A-W-Sp	S-A-W-Sp	A-W-Sp	W	W-Sp	A-W-Sp	S-A-W-Sp	A	S-A-W-Sp	S		A-W-Sp	W-Sp	S-A-W-Sp	S-A-W-Sp	A-W	W-Sp	S-A-W-Sp
Excess fluid		S-A-W-Sp	S-Sp	A-W-Sp	A-W-Sp	A	S-Sp	A	S-W-Sp	Sp	Sp	W-Sp	W-Sp	S-W-Sp	S-A-W-Sp	A-W	S-W-Sp	S-A-W-Sp
Excess mucus			S-A-W-Sp	S-A-W-Sp	W	S-A-W-Sp	S-A-W-Sp	A-W-Sp	S-A-W-Sp	S-A-W		S-A-W-Sp	V S-A-W-Sp	S-A-W-Sp	S-A-W-Sp	S-A-W-Sp	S-A-Sp	S-A-W-Sp
Litter intake				A-W-Sp		A-W-Sp	S-A-W-Sp	A-W	S-A-W-Sp	W	A-W-Sp	A-W-Sp	A-W-Sp	S-A-W-Sp	S-A-W-Sp	W-Sp	S-A-Sp	S-A-W-Sp
Mealworm intake				S					Sp	A							S	S
Thick intestine							Sp		W-Sp	Sp	Sp	W-Sp	W	W	A-W	S-A	S	W-Sp
Thin intestine							A-W-Sp	A-W-Sp	S-A-W-Sp	W		S-A-W-Sp	S-A-W-Sp	A-W-Sp	A-Sp	S-W-Sp	A	S-A-W-Sp
Passage feed								A-W-Sp	S-A-W-Sp	W		A-W-Sp	A-W-Sp	S-A-W-Sp	S-A-W-Sp	A-W-Sp	W-Sp	V S-A-W-Sp
Altered intestinal tone									S-A	W		S-A-W-Sp	A-Sp	A-Sp	S-A	A-Sp	A	A
Turkish towel										S-W		S-A-W-Sp	S-W-Sp	S-A-W-Sp	S-W-Sp	W-Sp	S-Sp	S-A-W-Sp
Worms											S-A	S-A	S	A		W		
Duodenitis											S-A-W-Sp	S-A-W-Sp	S-A-W-Sp	A-W-Sp	W	S-W-Sp	A-W-Sp	S-A-W-Sp
Enteritis														A-W-Sp	W-Sp	S-W-Sp	P	S-A-W-Sp
<i>Eimeria acervulina</i>																		
<i>Eimeria maxima</i>																		
<i>Eimeria tenella</i>																		
<i>Eimeria maxima</i> micro																		

Legend: S—Summer; A—Autumn; W—Winter; Sp—Spring.

(Sp). The statistical analyses for each season of the year are displayed in tables. **Table 2** shows a significant correlation between broiler's intestinal alterations parameters and summer season. **Table 3** depicts a statistical correlation between broiler's intestinal alterations traits and autumn season. In **Table 4** we observed a significant correlation between broiler's intestinal alterations traits and winter season. Whereas, **Table 5** indicates a significant correlation between broiler's intestinal alterations traits and spring season. The data considered in these analyses have a significance level of $P = 0.05$. Below, we will present the most notable findings obtained from the statistics presented in **Tables 2-5**. Food passage showed correlation with cell desquamation, excess mucus, and litter consumption across all four seasons ($P = 0.05$). During the spring season, there was a correlation between food transit and an abundance of fluid ($P = 0.0167$), as well as the presence of both thick ($P = 0.0375$) and thin ($P = 0.0061$) intestines. The winter and fall seasons exhibited a correlation with reduced intestinal thickness ($P = 0.0051$ and $P = 0.0009$, respectively), but the summer season showed a correlation with increased intestinal fluid levels ($P = 0.0001$). Throughout all seasons of the year, there was a clear positive relationship between *E. acervulina* and intestinal desquamation, excess mucus, bedding intake, food passage, and Turkish towel appearance ($P = 0.05$).

During autumn, winter, and spring, *E. acervulina* was found to have a significant association with duodenitis and overall enteritis ($P = 0.05$). Moreover, it is linked to the presence of worms throughout autumn ($P = 0.0465$).

There was a significant correlation between food intake and the shedding of cells, excessive production of mucus, and consumption of litter throughout the four seasons ($P = 0.05$).

E. maxima was consistently linked to intestinal scaling, elevated fluid levels, and mucus production throughout all four seasons ($P = 0.05$). *Eimeria maxima* had a significant correlation with bedding intake, food passage and Turkish towel appearance, while *Eimeria acervulina* only during the summer, winter, and spring seasons ($P = 0.05$). It is important to highlight that *E. maxima* is the sole species that is not associated with worm presence throughout any of the seasons.

Eimeria tenella had a significant correlation with increased mucus production during all four seasons ($P = 0.05$). Additionally, it was associated with inflammation of the duodenum and small intestine during the summer, winter, and spring ($P = 0.05$). Furthermore, there is a correlation between the existence of worms and this phenomenon ($P = 0.0154$). Additionally, there was a significant positive correlation between *E. tenella* and *E. acervulina* during the winter ($P = 0.0018$) and spring ($P = 0.0047$). Nevertheless, it is associated with *E. maxima* during the autumn ($P = 0.001$) and spring ($P = 0.0409$).

During the spring season, there was a significant correlation between *E. maxima* micro and *E. maxima* ($P = 0.0010$) as well as *E. tenella* ($P = 0.0036$). The association with *E. acervulina* is observed during the winter ($P = 0.0162$) and spring ($P = 0.0001$). *E. maxima* micro is linked to increased clinical signs in the spring, such as shedding of cells, excessive fluid and mucus, ingestion of bedding,

Table 2. Statistical analysis correlation between broiler's intestinal alterations traits and summer season.

Summer		Excess fluid	Excess mucus	Litter intake	Mealworm intake	Thick intestine	Thin intestine	Passage feed	Altered intestinal tone	Turkish towel	Worms	Necrotic enteritis	Duodenitis Enteritis	<i>Eimeria acervulina</i>	<i>Eimeria maxima</i>	<i>Eimeria tenella</i>	<i>Eimeria maxima</i> micro	Gizzard erosion
Cell desquamation	0.69061	0.73669	0.43856	0.43334	0.12474	0.21424	0.21424	0.89652	0.46020	0.87783	0.57363	0.31578	0.21886	0.74675	0.73961	0.35024	0.44578	0.89662
Excess fluid	0.0021	0.0007	0.0782	0.0823	0.6334	0.4090	0.4090	<0.0001	0.0631	<0.0001	0.0161	0.2169	0.3987	0.0006	0.0007	0.1681	0.0729	<0.0001
Excess mucus	0.68948	0.74634	0.39549	0.03172	0.40689	0.40689	0.40689	0.86361	0.20768	0.69210	0.11308	0.29071	0.33301	0.78547	0.85004	0.36407	0.53505	0.71079
Litter intake	0.0022	0.0006	0.1161	0.9038	0.1050	0.1050	0.1050	<0.0001	0.4238	0.0021	0.6656	0.2576	0.1915	0.0002	<0.0001	0.1508	0.0269	0.0014
Mealworm intake	0.57234	0.26602	0.11739	0.75453	0.79445	0.79445	0.79445	0.79445	0.42316	0.87438	0.54992	0.78559	0.74375	0.73317	0.59892	0.64156	0.52373	0.85893
Thick intestine	0.0164	0.3021	0.6536	0.0005	0.0005	0.0005	0.0005	0.0001	0.0906	<0.0001	0.0222	0.0002	0.0006	0.0008	0.0111	0.0055	0.0309	<0.0001
Thin intestine	0.26822	0.10975	0.47556	0.70224	0.10730	0.54104	0.54104	0.70224	0.10730	0.54104	-0.10260	0.39693	0.40680	0.58082	0.61531	0.25685	0.62717	0.63199
Passage feed	0.2979	0.6750	0.0537	0.0017	0.6819	0.0249	0.6952	0.0017	0.6819	0.0249	0.6952	0.11147	0.1051	0.0145	0.0086	0.3196	0.0070	0.0065
Altered intestinal tone	0.64861	0.04678	0.34144	0.34144	-0.14563	0.25346	-0.07383	0.34144	-0.14563	0.25346	-0.07383	0.06663	-0.0014	0.22422	0.37498	0.43748	0.49160	0.49656
Turkish towel	0.0049	0.8585	0.1798	0.5771	0.3263	0.7783	0.7783	0.1798	0.5771	0.3263	0.7783	0.7994	0.9957	0.3869	0.1381	0.0791	0.0450	0.0426
Worms	0.10819	0.00595	-0.07055	-0.01780	-0.19075	-0.19075	-0.19075	0.00595	-0.07055	-0.01780	-0.19075	0.14062	-0.00246	-0.16672	0.08968	0.68078	0.38680	0.37647
	0.6794	0.9819	0.7879	0.9459	0.4633	0.4633	0.4633	0.9819	0.7879	0.9459	0.4633	0.5904	0.9925	0.5225	0.7321	0.0026	0.1251	0.1364
	0.37871	0.30411	0.52548	0.32575	0.32575	0.32575	0.32575	0.37871	0.30411	0.52548	0.32575	0.91430	0.90878	0.46891	0.25614	0.55913	0.32223	0.50589
	0.1339	0.2353	0.0303	0.2020	0.2020	0.2020	0.2020	0.1339	0.2353	0.0303	0.2020	<0.0001	<0.0001	0.0576	0.3210	0.0196	0.2072	0.0383
	0.32348	0.83785	0.33639	0.33639	0.33639	0.33639	0.33639	0.32348	0.83785	0.33639	0.33639	0.35918	0.32833	0.85695	0.77322	0.27634	0.50190	0.86330
	0.2053	<0.0001	0.1868	0.1868	0.1868	0.1868	0.1868	0.2053	<0.0001	0.1868	0.1868	0.1568	0.1982	<0.0001	0.0003	0.2830	0.0401	<0.0001
	0.60334	0.61855	0.61855	0.61855	0.61855	0.61855	0.61855	0.60334	0.61855	0.61855	0.61855	0.48829	0.35905	0.34320	0.58272	0.28302	0.04064	0.45529
	0.0103	0.0081	0.0081	0.0081	0.0081	0.0081	0.0081	0.0103	0.0081	0.0081	0.0081	0.0467	0.1570	0.1775	0.0141	0.2710	0.8769	0.0663
	0.69053	0.58624	0.78826	0.78826	0.78826	0.78826	0.78826	0.69053	0.58624	0.78826	0.78826	0.65321	0.58624	0.78826	0.73100	0.43913	0.53301	0.88449
	0.0022	0.0134	0.0002	0.0002	0.0002	0.0002	0.0002	0.0022	0.0134	0.0002	0.0002	0.0045	0.0134	0.0002	0.0009	0.0778	0.0276	<0.0001
	0.53394	0.47688	0.39418	0.25716	0.25507	0.47216	0.47216	0.53394	0.47688	0.39418	0.25716	0.53394	0.47688	0.39418	0.25716	0.25507	0.00610	0.47216
	0.0273	0.0529	0.1174	0.3190	0.3231	0.9814	0.0557	0.0273	0.0529	0.1174	0.3190	0.0273	0.0529	0.1174	0.3190	0.3231	0.9814	0.0557

Continued

Necrotic enteritis
Duodenitis	0.90266	0.45140	0.27802	0.61094	0.32627	0.58559					
Enteritis	<0.0001	0.0689	0.2799	0.0092	0.2012	0.0135					
<i>Eimeria acervulina</i>		0.42339	0.22320	0.53657	0.42882	0.47205					
<i>Eimeria tenella</i>		0.0904	0.3892	0.0264	0.0859	0.0557					
<i>Eimeria maxima</i>			0.61495	0.23670	0.31689	0.73321					
<i>Eimeria maxima</i>			0.0086	0.3604	0.2152	0.0008					
<i>Eimeria maxima</i>				0.36200	0.45114	0.71387					
<i>Eimeria maxima</i>				0.1533	0.0691	0.0013					
<i>Eimeria maxima</i>					0.52985	0.62456					
<i>Eimeria maxima</i>					0.0287	0.0074					
<i>Eimeria maxima</i>						0.62158					
<i>Eimeria maxima</i>						0.0077					

Table 3. Statistical analysis correlation between broiler’s intestinal alterations traits and autumn season.

Autumn		Excess fluid	Excess mucus	Litter intake	Mealworm intake	Thick intestine	Thin intestine	Passage feed	Altered intestinal tone	Turkish towel	Worms	Necrotic enteritis	Duodenitis	Enteritis	<i>Eimeria acervulin</i> _a	<i>Eimeria maxima</i>	<i>Eimeria tenella</i>	<i>Eimeria maxima</i> micro	Gizzard erosion
Cell desquamation	0.75318	0.71777	0.56042	0.50907	0.30003	0.67396	0.70578	0.62033	0.52943	0.42339	-0.07959	0.47180	0.37314	0.65842	0.62412	0.71795	0.27387	0.82403	
Excess fluid	0.0002	0.0005	0.0126	0.0260	0.2120	0.0016	0.0007	0.0046	0.0197	0.0709	0.7460	0.0414	0.1156	0.0022	0.0043	0.0005	0.2566	<0.0001	
Excess mucus	0.49950	0.25824	0.24117	0.24117	0.46250	0.48976	0.37491	0.53289	0.22227	0.05150	0.28099	0.08733	0.18911	0.38958	0.85364	0.79429	0.17097	0.50257	
Litter intake	0.0294	0.2858	0.3199	0.3199	0.0462	0.0333	0.1138	0.0188	0.3604	0.8342	0.2439	0.7222	0.4381	0.0992	<0.0001	<0.0001	0.4840	0.0283	
Mealworm intake	0.72191	0.26313	0.28089	0.28089	0.93760	0.93760	0.74326	0.88756	0.56140	0.46076	-0.11811	0.87628	0.81760	0.65705	0.59370	0.62966	0.51751	0.91915	
Thin intestine	0.0005	0.2764	0.2441	0.2441	<0.0001	<0.0001	0.0003	<0.0001	0.0124	0.0471	0.6301	<0.0001	<0.0001	0.0022	0.0074	0.0039	0.0232	<0.0001	
Passage feed	-0.06656	0.02871	0.77812	0.77812	0.84657	0.84657	0.84657	0.58871	0.74506	0.15673	-0.18588	0.79197	0.55720	0.74549	0.32875	0.35875	0.53686	0.84095	
Altered intestinal tone	0.7866	0.9071	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0080	0.0003	0.5217	0.4461	<0.0001	0.0132	0.0002	0.1694	0.1315	0.0178	<0.0001	
Turkish towel	0.08033	0.20575	0.25788	0.25788	0.20803	0.28806	0.28806	0.20803	0.28806	0.66581	-0.14318	0.11485	0.05536	0.36651	0.12443	0.28969	0.02280	0.27454	
Worms	0.7437	0.3981	0.2865	0.2865	0.3928	0.2317	0.0019	0.5587	0.6397	0.8219	0.1227	0.6118	0.2290	0.9262	0.59431	0.08863	0.16425	0.2553	
Excess fluid	0.29294	0.01289	0.01289	0.01289	0.24359	-0.22518	-0.07320	-0.12332	-0.01117	0.02503	0.04041	0.62567	0.59431	0.08863	0.16425	0.5016	0.88741	0.83495	
Excess mucus	0.2236	0.9582	0.3149	0.3149	0.84996	0.57895	0.41209	-0.03909	0.85079	0.64656	0.66062	0.64053	0.59795	0.66637	0.88741	0.88741	0.88741	0.88741	
Litter intake	0.69903	0.0009	0.0009	0.0009	<0.0001	0.0094	0.0796	0.8738	<0.0001	0.0028	0.0021	0.0031	0.0068	0.0018	<0.0001	<0.0001	<0.0001	<0.0001	
Mealworm intake	0.61689	0.71410	0.27671	0.27671	0.70611	0.53514	0.70187	0.41089	0.51546	0.42092	0.83495	0.0239	0.0727	0.59079	0.74076	0.74076	0.74076	0.74076	
Thin intestine	0.0049	0.0006	0.2515	0.2515	0.6920	0.0007	0.0182	0.0008	0.0805	0.0239	0.0727	0.59079	0.74076	0.74076	0.74076	0.74076	0.74076	0.74076	
Passage feed	0.56350	0.0120	0.3423	0.3423	0.4916	0.0007	0.0032	0.0111	0.0170	0.0176	0.0077	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	
Altered intestinal tone	0.26440	-0.25464	0.61835	0.61835	0.39255	0.77080	0.07822	0.06069	0.31139	0.68375	0.68375	0.68375	0.68375	0.68375	0.68375	0.68375	0.68375	0.68375	
Turkish towel	0.2740	0.2928	0.0048	0.0048	0.0964	0.0001	0.7503	0.8051	0.1944	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	0.0012	
Worms	-0.01634	0.46399	0.38900	0.38900	0.46183	0.08513	0.26601	0.08167	0.44628	0.44628	0.44628	0.44628	0.44628	0.44628	0.44628	0.44628	0.44628	0.44628	
Excess fluid	0.9471	0.0454	0.0997	0.0997	0.0465	0.7289	0.2710	0.7396	0.0555	0.0555	0.0555	0.0555	0.0555	0.0555	0.0555	0.0555	0.0555	0.0555	

Continued											
Necrotic enteritis	-0.17746	-0.12136	-0.14946	0.36328	0.15412	-0.13159	-0.09746				
	0.4673	0.6207	0.5414	0.1263	0.5287	0.5913	0.6914				
Duodenitis		0.82162	0.61732	0.23562	0.26044	0.51806	0.86301				
		<0.0001	0.0049	0.3315	0.2815	0.0231	<0.0001				
Enteritis			0.49505	0.24230	0.34436	0.28055	0.72699				
			0.0312	0.3176	0.1488	0.2447	0.0004				
<i>Eimeria acervulina</i>				0.24467	0.39381	0.36931	0.77220				
				0.3127	0.0953	0.1197	0.0001				
<i>Eimeria maxima</i>					0.85733	0.33136	0.51190				
					<0.0001	0.1658	0.0251				
<i>Eimeria tenella</i>						0.29974	0.57100				
						0.2125	0.0107				
<i>Eimeria maxima</i> micro							0.47166				
							0.0415				

Table 4. Statistical analysis correlation between broiler’s intestinal alterations traits and winter season.

Winter		Excess fluid	Excess mucus	Litter intake	Mealworm intake	Thick intestine	Thin intestine	Passage feed	Altered intestinal tone	Turkish towel	Worms	Necrotic enteritis	Duodenitis	Enteritis	<i>Eimeria acervulina</i>	<i>Eimeria maxima</i>	<i>Eimeria tenella</i>	<i>Eimeria maxima</i> micro	Gizzard erosion	
Pearson Correlation Coefficients, N = 18		Prob > r under H0: Rho= 0																		
Cell desquamation	0.72323	0.64387	0.71599	0.26354	0.64174	0.58089	0.75942	0.28781	0.84857	0.30271	0.17872	0.66491	0.83741	0.79804	0.87108	0.63463	0.65356	0.91945		
Excess fluid	0.0079	0.0239	0.0088	0.4079	0.0245	0.0476	0.0042	0.3643	0.0005	0.3389	0.5784	0.0183	0.0007	0.0019	0.0002	0.0266	0.0212	<0.0001		
Excess mucus	0.59146	0.0428	0.0517	0.1067	0.69183	0.54293	0.35270	0.12892	0.69821	0.19885	0.46472	0.73141	0.67865	0.70835	0.63311	0.70193	0.67833	0.78861		
Litter intake	0.85581	0.0004	0.85581	0.06598	0.69563	0.93983	0.76327	0.76480	0.68809	0.62557	0.35582	0.94264	0.92774	0.89646	0.62154	0.86785	0.47397	0.79052		
Mealworm intake	0.0004	0.8386	0.0004	0.8386	0.0120	<0.0001	0.0039	0.0038	0.0134	0.0296	0.2563	<0.0001	<0.0001	<0.0001	0.0310	0.0003	0.1196	0.0022		
Thick intestine	-0.01972	0.42511	0.95372	0.79718	0.70647	0.73896	0.79718	0.70647	0.73896	0.74548	0.23001	0.74117	0.84568	0.80964	0.64838	0.85051	0.54994	0.68526		
Thin intestine	0.9515	0.1683	<0.0001	0.0019	0.0102	0.0060	0.0019	0.0102	0.0060	0.0054	0.4720	0.0058	0.0005	0.0014	0.0226	0.0005	0.0640	0.0139		
Passage feed	0.42640	0.1669	0.9248	0.9836	0.7075	0.0931	0.7075	0.7075	0.0931	0.7271	0.7787	0.4631	0.8629	0.9403	0.4813	0.4839	0.4542	0.4177		
Altered intestinal tone	0.47793	0.47692	0.47692	0.55594	0.47692	0.70972	0.55594	0.47692	0.70972	0.24978	0.10660	0.85150	0.67762	0.71502	0.65404	0.55775	0.51327	0.79840		
Turkish towel	0.1161	0.1169	0.1169	0.0605	0.1169	0.0097	0.0605	0.1169	0.0097	0.4336	0.7416	0.0004	0.0155	0.0090	0.0210	0.0595	0.0879	0.0018		
Worms	0.74886	0.77967	0.77967	0.74886	0.77967	0.65065	0.74886	0.77967	0.65065	0.75057	0.30722	0.83115	0.85609	0.82172	0.54827	0.87058	0.48765	0.65371		
	0.0051	0.0028	0.0028	0.0051	0.0028	0.0220	0.0051	0.0028	0.0220	0.0049	0.3314	0.0008	0.0004	0.0010	0.0649	0.0002	0.1078	0.0211		
	0.68869	0.76246	0.76246	0.68869	0.76246	0.64186	0.68869	0.76246	0.64186	-0.13990	0.71086	0.71086	0.78432	0.75504	0.71426	0.58968	0.58845	0.72808		
	0.0133	0.0039	0.0039	0.0133	0.0039	0.0244	0.0133	0.0039	0.0244	0.6645	0.0096	0.0096	0.0025	0.0045	0.0091	0.0436	0.0441	0.0073		
	0.50162	0.79560	0.79560	0.50162	0.79560	-0.16103	0.50162	0.79560	-0.16103	0.79560	-0.16103	0.64477	0.56649	0.54906	0.39178	0.57262	0.18616	0.34610		
	0.0966	0.0020	0.0020	0.0966	0.0020	0.6171	0.0966	0.0020	0.6171	0.0020	0.6171	0.0236	0.0548	0.0645	0.2079	0.0517	0.5624	0.2705		
	0.65226	-0.04017	0.73670	0.65226	-0.04017	0.73670	0.65226	-0.04017	0.73670	0.65226	-0.04017	0.73670	0.71288	0.67584	0.72298	0.74381	0.56573	0.77518		
	0.0215	0.9014	0.0063	0.0215	0.9014	0.0063	0.0215	0.9014	0.0063	0.0215	0.9014	0.0063	0.0093	0.0158	0.0079	0.0055	0.0552	0.0031		
	-0.18796	0.52038	0.42395	-0.18796	0.52038	0.42395	-0.18796	0.52038	0.42395	-0.18796	0.52038	0.42395	0.39858	0.39858	0.28888	0.67783	0.20144	0.25242		
	0.5586	0.0828	0.1696	0.5586	0.0828	0.1696	0.5586	0.0828	0.1696	0.5586	0.0828	0.1696	0.1994	0.3625	0.0154	0.5301	0.4286	0.4286		

Continued										
Necrotic enteritis	0.33737	0.40744	0.43841	0.09014	0.50710	0.10965	0.38740			
	0.2835	0.1886	0.1540	0.7805	0.0924	0.7344	0.2134			
Duodenitis		0.87562	0.90310	0.61969	0.83182	0.61063	0.84753			
		0.0002	<0.0001	0.0316	0.0008	0.0349	0.0005			
Enteritis			0.94611	0.77238	0.79352	0.56823	0.91628			
			<0.0001	0.0032	0.0021	0.0539	<0.0001			
<i>Eimeria acervulina</i>				0.72819	0.80009	0.67411	0.91022			
				0.0072	0.0018	0.0162	<0.0001			
<i>Eimeria maxima</i>					0.55253	0.50489	0.84430			
					0.0625	0.0941	0.0006			
<i>Eimeria tenella</i>						0.44423	0.72970			
							0.1480			0.0071
<i>Eimeria maxima</i> micro										0.66029
										0.0194

Table 5. Statistical analysis correlation between broiler's intestinal alterations traits and spring season.

Spring	Excess fluid	Excess mucus	Litter intake	Mealworm intake	Thick intestine	Thin intestine	Passage feed	Altered intestinal tone	Turkish towel	Worms	Necrotic enteritis	Duodenitis	Enteritis	<i>Eimeria acervulina</i>	<i>Eimeria maxima</i>	<i>Eimeria tenella</i>	<i>Eimeria maxima</i> micro	Gizzard erosion
Cell desquamation	0.85814	0.70504	0.57921	0.38311	0.74791	0.50595	0.72646	0.15427	0.60260	0.48150	0.47680	0.55773	0.59815	0.62343	0.85756	0.44242	0.65117	0.90787
Excess fluid	<0.0001	0.0023	0.0187	0.1430	0.0009	0.0455	0.0014	0.5684	0.0135	0.0590	0.0619	0.0248	0.0144	0.0099	<0.0001	0.0862	0.0063	<0.0001
Excess mucus	0.54012	0.56535	0.58735	0.35635	0.89583	0.38039	0.58735	0.01303	0.58791	0.40717	0.80570	0.52534	0.58667	0.54286	0.67192	0.28402	0.66566	0.73442
Litter intake	0.0308	0.0225	0.0167	0.1755	<0.0001	0.1461	0.0167	0.9618	0.0166	0.1175	0.0002	0.0366	0.0169	0.0298	0.0044	0.2864	0.0049	0.0012
Mealworm intake	0.66260	0.08589	0.80125	0.08589	0.48926	0.93127	0.80125	0.53299	0.67127	0.16889	0.19554	0.80676	0.82595	0.83120	0.71764	0.60062	0.57344	0.90350
Thick intestine	0.0052	0.7518	0.0002	0.0227	0.0544	<0.0001	0.0002	0.0335	0.0044	0.5318	0.4680	0.0002	<0.0001	<0.0001	0.0017	0.0139	0.0202	<0.0001
Thin intestine	0.56446	0.47678	0.60034	0.56446	0.47678	0.60034	0.81667	0.45825	0.92284	0.25752	0.24033	0.84659	0.62605	0.88740	0.61625	0.70701	0.87962	0.75679
Passage feed	0.0227	0.0619	0.0139	0.0227	0.0619	0.0139	0.0001	0.0742	<0.0001	0.3356	0.3699	<0.0001	0.0095	<0.0001	0.0110	0.0022	<0.0001	0.0007
Altered intestinal tone	0.33701	0.2018	0.7303	0.33701	0.2018	0.7303	0.43312	-0.13534	0.53624	0.46174	0.04309	0.39339	0.09240	0.33438	0.21934	0.33902	0.48210	0.34457
Turkish towel	0.2018	0.7303	0.0938	0.6173	0.0323	0.0718	0.0938	0.6173	0.0323	0.0718	0.8741	0.1317	0.7336	0.2056	0.4144	0.1990	0.0586	0.1912
Worms	0.33596	0.52322	0.52322	-0.06336	0.50561	0.63478	0.52322	-0.06336	0.50561	0.63478	0.79436	0.52007	0.48664	0.47247	0.47664	0.28386	0.50253	0.65407
	0.2033	0.0375	0.0375	0.8157	0.0457	0.0082	0.0375	0.8157	0.0457	0.0082	0.0002	0.0389	0.0559	0.0646	0.0620	0.2867	0.0473	0.0060
	0.65341	0.0061	0.0061	0.53334	0.61485	-0.01552	0.65341	0.53334	0.61485	-0.01552	0.10485	0.71811	0.72422	0.80546	0.62572	0.43969	0.48503	0.75109
	0.0061	0.0334	0.0334	0.0334	0.0113	0.9545	0.0061	0.0334	0.0113	0.9545	0.6992	0.0017	0.0015	0.0002	0.0095	0.0883	0.0569	0.0008
	0.61337	0.77348	0.77348	0.61337	0.77348	0.39575	0.77348	0.61337	0.77348	0.39575	0.23678	0.86024	0.80343	0.81705	0.77049	0.77842	0.74829	0.84852
	0.0115	0.0004	0.0115	0.0115	0.0004	0.1292	0.0115	0.0115	0.0004	0.1292	0.3773	<0.0001	0.0002	0.0001	0.0005	0.0004	0.0009	<0.0001
	0.27416	0.27416	0.27416	0.27416	0.27416	-0.14421	0.27416	0.27416	0.27416	-0.14421	-0.05355	0.57108	0.64837	0.50999	0.42739	0.78706	0.45488	0.40654
	0.3042	0.5941	0.5941	0.3042	0.5941	0.8439	0.3042	0.5941	0.8439	0.8439	0.0209	0.0066	0.0436	0.0987	0.0003	0.0003	0.0767	0.1181
	0.26309	0.18837	0.18837	0.26309	0.18837	0.74608	0.26309	0.18837	0.74608	0.74608	0.52354	0.52354	0.88274	0.61185	0.52058	0.84542	0.84542	0.75349
	0.3249	0.4848	0.4848	0.3249	0.4848	0.0009	0.3249	0.4848	0.0009	0.3249	0.0374	0.0374	<0.0001	0.0118	0.0387	<0.0001	<0.0001	0.0008
	0.31203	0.35880	0.35880	0.31203	0.35880	0.18979	0.31203	0.35880	0.18979	0.18979	0.09926	0.09926	0.17698	0.23745	0.10161	0.23745	0.10161	0.38595
	0.2394	0.1723	0.1723	0.2394	0.1723	0.4814	0.2394	0.1723	0.4814	0.4814	0.7146	0.7146	0.5120	0.3759	0.7081	0.3759	0.7081	0.1398

Continued										
Necrotic enteritis	0.30664	0.46489	0.16357	0.26015	0.05250	0.30881	0.33313			
	0.2480	0.0696	0.5450	0.3305	0.8469	0.2445	0.2074			
Duodenitis		0.87146	0.75873	0.49127	0.72217	0.63327	0.79850			
		<0.0001	0.0007	0.0533	0.0016	0.0085	0.0002			
Enteritis			0.62351	0.57872	0.62505	0.51554	0.76406			
			0.0099	0.0188	0.0096	0.0410	0.0006			
<i>Eimeria acervulina</i>				0.75050	0.66728	0.84232	0.81157			
				0.0008	0.0047	<0.0001	0.0001			
<i>Eimeria maxima</i>					0.51574	0.74054	0.81465			
					0.0409	0.0010	0.0001			
<i>Eimeria tenella</i>						0.68251	0.63764			
						0.0036	0.0079			
<i>Eimeria maxima</i> micro							0.72340			
							0.0015			

passage of food, Turkish towel appearance, duodenitis, and enteritis ($P = 0.05$).

The presence of gizzard erosion was observed to be associated with the following signs throughout all four seasons: shedding of intestinal cells, excessive fluid and mucus, ingestion of bedding material, intestinal thinning, food passage, presence of Turkish towel appearance, duodenal inflammation, and the four *Eimeria* species. Nevertheless, there was a correlation between the consumption of mealworms and the erosion of the gizzard during the summer ($P = 0.0426$). Otherwise, during the spring and winter, there was a significant association between mealworm intake and thickening of the gut ($P = 0.0060$; $P = 0.0018$). In fall, there was a significant correlation with altered intestinal tone ($P = 0.0003$), and in autumn, winter, and spring, there was a correlation with enteritis ($P = 0.05$). The aforementioned results are summarized in **Table 1**. Additionally, *E. maxima* micro and *E. acervulina* exhibited higher prevalence rates (mean) during the winter (52.83% and 26.42% respectively). *E. maxima* also had a prevalence of 11.31% in the spring, while *E. tenella* had a prevalence of 6.74% in the autumn (**Table 6**). Hence, the presence of *E. maxima* microorganisms was observed to be higher during the winter season compared to the summer season ($P = 0.0491$). No significant variations were observed in the occurrence of other *Eimeria* species across different seasons (**Table 6**).

The results analysis revealed a correlation between *Eimeria acervulina* and *Eimeria maxima* during the summer, winter, and spring seasons. During the winter and spring seasons, a co-occurrence of *Eimeria acervulina* with *Eimeria tenella* was observed, while a co-occurrence of *Eimeria acervulina* with *Eimeria maxima* micro occurred during the same seasons. *Eimeria maxima* exhibits a correlation with *Eimeria acervulina*, as well as a correlation with *Eimeria tenella* in the autumn and spring, and with *Eimeria maxima* micro in the spring. Furthermore, *E. tenella* shows a correlation with *E. maxima* micro in the spring, in addition to the aforementioned correlations.

4. Discussion

The occurrence of necrotic enteritis in the spring was positively associated with an abundance of fluid in the last portion of the intestine. However, there was no significant link between specific necrotic enteritis and the presence of *E. acervulina*, *E. tenella*, *E. maxima*, and *E. maxima* micro. Santiani *et al.* [10] observed

Table 6. Seasonal variation in the prevalence of *Eimeria* infection (%).

	Spring	Summer	Autumn	Winter	P-Value
<i>E. acervulina</i>	22.12	13.70	18.10	26.42	0.1878
<i>E. maxima</i>	11.31	8.29	10.79	7.67	0.5696
<i>E. maxima</i> micro	49.50 ^{AB}	25.88 ^B	41.63 ^{AB}	52.83 ^A	0.0491
<i>E. tenella</i>	6.37	2.50	6.74	6.25	0.1896

Means followed by different letters on the lines differ by Tukey's test (5%).

that 93.8% of the studied batches with subclinical necrotic enteritis signs tested positive for coccidiosis. The macroscopic lesions of coccidiosis were classified as grade 1 for *E. acervulina* (27%), *E. tenella* (9.7%), and *E. maxima* (8.9%). The histopathological examination revealed no necrotic tissue, but instead showed bleeding in the mucosa and submucosa, along with the presence of *Eimeria* spp. The absence of *Clostridium perfringens* type A netB+ indicates that the macroscopic lesions primarily observed in the jejunum were not indicative of necrotic enteritis, as confirmed by histology and the absence of the NetB gene. This finding aligns with our correlation analysis, which indicates that necrotic enteritis is not correlated with *E. acervulina*, *E. tenella*, *E. maxima*, and *E. maxima* micro. There was a consistent relationship observed between gizzard erosion and intestinal alterations in broiler chickens over the majority of seasons throughout the year. Roppa *et al.* [11] reported that Aviadenovirus, particularly type 1, is present in 75% of asymptomatic birds, and this is associated with the occurrence of Gizzard Erosion Syndrome (GES). It is worth mentioning that, apart from gizzard erosion, additional alterations occur in the gastrointestinal system of birds across the seasons. The intake of mealworms has been associated with the occurrence of fine intestine, *E. maximum* micro, and gizzard erosion throughout the summer. In autumn, the presence of worms was correlated with gizzard erosion, and in spring, the presence of Turkish towel appearance was associated with all these conditions. The mealworm undergoes its reproductive cycle, from incubation to adulthood, throughout a span of 55 days at a temperature of 27°C [12]. Cold temperatures (below 16.5°C) might effectively help control these insects by preventing the development of their immature stages, resulting in a drop in population [13]. During the warmer seasons, there is an increased abundance of insects, leading to a higher likelihood of birds consuming them and therefore experiencing gizzard erosion. Goodwin and Waltman [14] collected darkness beetles (*Aplhitobius diaperinus*) from seven premises (farms). Almost all picked *Aplhitobius diaperinus* were shown to contain myriad infectious organisms including dangerous bacteria like *Salmonella* spp., viruses (e.g., reovirus), and *Eimeria* (the causative agents of intestinal coccidiosis). The correlation observed between *E. maxima* micro and summer season could be explained by the phenomenon aforementioned by Goodwin and Waltman [14].

During different seasons, *E. acervulina* was most prevalent in winter, *E. maxima* in spring, and *E. tenella* in fall. The prevalence of *E. acervulina*, *E. maxima*, and *E. tenella* during the course of the year was found to be 13.45% in winter, 13.27% in spring, 11.88% in autumn, and 8.16% in summer. According to the findings of Ahad *et al.* [5], coccidiosis is most prevalent during the fall season, with a prevalence rate of 45.12%. It is less prevalent during the summer (30.84%), spring (23.81%), and winter (20.29%) seasons. Razmi and Kalideri [4] found that the occurrence of subclinical coccidiosis in broiler chickens in Iran was more common throughout the spring and winter seasons than during the fall or summer seasons. Based on our research conducted in Brazil, the prevalence of subclinical coccidiosis was highest during winter (52.83%), followed by

spring (49.5%), autumn (41.63%), and summer (25.88%). In their study, Awais and Akhtar [15] found that the condition had the highest prevalence during the fall season (60.02%), followed by summer (47.42%), spring (36.92%), and winter (29.89%). Seasonal variation in the prevalence of *Eimeria* infection may be attributed primarily to the diverse effects of each season in the evaluated geographical locations, rather than to intrinsic factors of the disease itself. Differences in environmental humidity are likely a key factor contributing to the variations in incidences observed. The high occurrence of rainfall during the winter, spring, and autumn seasons may contribute to the higher incidence observed. Increased humidity facilitates the proliferation of oocysts by creating a more favorable environment for their sporulation and survival in the litter [4]. The occurrence of this disease throughout the year is influenced by both relative humidity and environmental temperature, which directly affects the oocyst sporulation rate [15]. Bachaya *et al.* [16] found that the occurrence of coccidiosis decreases when the surrounding humidity diminishes. Due to the increased relative humidity during the rainfall months, it has been found that the maximum occurrence of this phenomenon takes place in Pakistan in September, October, December, and January.

In a study conducted by Schneiders *et al.* [17], it was discovered that heat stress led to a decrease in the release of *E. maxima* oocysts in birds. Schneiders *et al.* [17] found that this decrease is associated with reduced expression of genes involved in gamete fusion and replication. The decreased prevalence reported in our summer survey (25.88%) can be attributed to this feature.

According to Taylor *et al.* [18], the most reliable indicators of *Eimeria* infection are the intestinal lesions. Avian coccidiosis is recognized for its ability to reduce body weight gain, deteriorate feed conversion, and increase crypt depth within the small intestine [19]. During the whole life cycle of the parasite, each oocyst has the potential to harm 2048 enterocytes [9]. Galli *et al.* [2] have been shown that coccidiosis disrupts the intestinal energy balance by reducing ATP levels, attributed to an increase in the production of reactive oxygen species (ROS). Coccidiosis enhances intestinal permeability, upregulates claudin junction protein expression, and concurrently decreases villus height by 20% [20]. These alterations facilitate the entry of viruses and toxins into the intestinal lumen, impeding nutrient absorption and stimulating intestinal mucus production. Consequently, modifications occur in the gastrointestinal system of birds, resulting in a decrease in food absorption. Indeed, coccidiosis induces an increase in the rate of intestinal protein metabolism, leading to significant energy expenditure as a consequence of inflammation. Previously, Gazoni *et al.* [9] established a correlation between *E. maxima* and *E. acervulina* and the occurrence of gizzard erosion; potentially due to broiler chickens ingesting a greater amount of bedding material. Gastrointestinal diseases commonly lead to an elevated rate of nutrient transit. This phenomenon arises due to inflammation in the intestines, resulting in excessive mucus production. As a result, enterocytes struggle to absorb nutrients effectively. Therefore, it is crucial to emphasize the

significance of examining intestinal abnormalities in the field and understanding their association with coccidiosis and other diseases. The insights gained from this study would be valuable in developing customized control strategies and preventive programs for managing coccidiosis in poultry, particularly in regions with similar climates and poultry production systems.

5. Conclusion

Seasonal variations in coccidiosis incidence showed a clear correlation, with statistically significant data observed, typically at least $P = 0.05$. From 2012 to 2018, *E. maxima* micro and *E. acervulina* were prevalent during the winter, *E. maxima* during the spring, and *E. tenella* during the autumn. Furthermore, regardless of the season, *Eimeria maxima* micro was found to be more prevalent in the Brazilian poultry industry compared to other *Eimeria* species. The presence of *Eimeria* species led to lesions that affected various factors related to the health of the bird's intestines, including shedding of intestinal tissue, erosion of the gizzard, digestive system transit rate, and excessive mucus production, among other effects. Therefore, these statistics are valuable for experts in the poultry industry to develop different strategies for managing this disease based on seasonal variations, thus minimizing financial losses.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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