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Evaluation of weight gain, feed intake, feed conversion and oocyst shedding of Eimeria maxima and Eimeria acervulina in broiler chickens

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ABSTRACT

The purpose of the present study was to evaluate the day and the time of sample collection of an experimental challenge with *Eimeria maxima* (EM) and *Eimeria acervulina* (EA) in broiler chickens. One day old male Cobb-Vantress broiler chickens were randomly allocated to one of three groups with ten replicates (n=8 chickens/replicate). Chickens were placed in battery cages with a controlled age-appropriate environment: Group 1) Negative control (no challenge or treatment); 2) Challenge control (*Eimeria* challenge only); 3) Challenge + Salinomycin. Challenged chickens were orally gavaged with the mixed culture of EM/EA (10,000 sporulated EM containing 4% wild-type EA) at 14 days of age. Performance parameters were recorded at days 7, 14, 20, and 23. Lesions scores were recorded post-mortem on days 20 and 23. Oocyst per gram (OPG) was performed on days six, seven, and eight post-challenge, and samples were collected at 9:00 AM and 6:00 PM on each day, respectively. Oocyst counts were significantly different ($P < 0.05$) between morning and afternoon on day six post coccidia challenge. The results of this study show that the day and the time at which samples are collected can have a significant effect on the reliability and validity of data.

Keywords: *Eimeria maxima*; *Eimeria acervulina*; oocysts shedding; performance parameters

Table of Contents

List of Tables

Chapter I. Introduction

Coccidiosis presently proves to be a major and pressing protozoan disease in the poultry industry worldwide (Dalloul and Lillehoj, 2006). Coccidiosis is caused by a protozoan parasite from the genus *Eimeria*. The life cycle of coccidial parasites includes asexual and sexual replication stages and begins when a bird ingests sporulated oocysts from the environment, as described by Conway and McKenzie (2007). After ingestion, four sporocysts contained in a single sporulated oocyst release two sporozoites. The release of the sporozoites is caused by digestive activity within the chicken. Released sporozoites will then "invade epithelial cells in a specific zone of the intestine or ceca", which is dependent on the *Eimeria* species (Chapman, 2003). Within the cell, sporozoites become trophozoites and feed for twelve to forty-eight hours to grow and eventually asexually divide via schizogony, or merogony; this stage is known as a schizont or meront. Within the parasite, the merozoite stages form and are released after the schizont matures and ruptures, which takes three days. This first generation of merozoites will invade more epithelial cells and repeat the multiplication process. The second generation of merozoites may induce a third schizogony cycle; this too is dependent on the *Eimeria* species. Both male (microgametocytes) and female (macrogametocytes) gametocytes will form. Macrogametocytes will grow into macrogametes. Microgametocytes will mature, rupture, and release biflagellate microgametes that fertilize the female macrogametes. Following fertilization, a "thickened wall forms around the macrogamete, forming a zygote" (Conway and McKenzie, 2007; McDougald and Fitz-Coy, 2013). At the conclusion of this cycle, a new oocyst is formed and will pass through the bird's droppings after rupturing its host cell (Tewari and Maharana, 2011).

Eimeria spp. oocysts, from a single or several simultaneous infections, are excreted in feces over a period of several days. Oocysts shedding starts low, reaches a plateau, and then decreases until the disease runs its course (Clarke, 1979; Williams, 1973). Interestingly, several investigators have reported that oocyst counts differ between morning and evening sampling collections (Hudman et al., 2000; Brown et al., 2001). This variability has been recognized for several years but has largely been overlooked (Misof, 2004). Recently, however, it has been shown that, the day post-inoculation and the time of day at which samples are collected can have a significant effect on the reliability and validity of the data (Brawner and Hill, 1999). Hence, the purpose of the present study was to evaluate the influence of oocyst shedding variation and day of sampling on experimental challenge with *Eimeria maxima* and *Eimeria acervulina* in broiler chickens.

Chapter II. Literature Review

Introduction: The Coccidiosis Challenge in Modern Broiler Production

As poultry rearing practices have changed to suit the modern climate, challenging diseases such as coccidiosis have presented themselves. As stated by Dalloul and Lillehoj (2006), coccidiosis is a protozoan disease caused by parasites from the genus *Eimeria*. Broilers faced with coccidiosis can experience malabsorption, inefficient feed utilization, impaired growth rate and mortality. Flocks enduring an outbreak of coccidiosis may also be left vulnerable to secondary infections (Ritzi et al., 2016). As a result, the poultry industry is burdened with an annual loss of over US $3 billion worldwide, much of which is allocated to in-feed medication and prevention (Dalloul and Lillehoj, 2006). While traditional methods of treating and preventing coccidiosis outbreaks have involved good management practices coupled with antibiotic feed additives, these methods have become a source of criticism in the commercial poultry industry (Ritzi et al., 2016). As a result, certain antibiotics have been banned in the European Union, leading to a "general decline in animal health with increased incidences of enteric conditions" (Ritzi et al., 2016). Overall, there has been an increase in pressure to find suitable alternatives that have similar or greater efficacy. The likelihood of modern research developing these effective alternatives hinges entirely on the understanding of the diseases that the industry must combat.

Chemotherapy and Emerging Drug Resistance

One common practice in managing coccidiosis is the use of prophylactic drugs and antimicrobials that inhibit the development of sporozoites/merozoites (Shivaramaiah et al., 2014). Ionophores are one such material that disrupt these stages of the life cycle through the

disruption of membrane integrity by “binding to cations and interfering with osmotic potential” (Shivaramaiah et al., 2014). Sulfonamides are additional anticoccidial agents that have been shown to control infection and boost immunity when administered (Shivaramaiah et al., 2014). However, there have been increasing concerns regarding emerging drug resistant strains of *Eimeria* in poultry production. A study reported by Bafundo and coworkers (2008) demonstrates a clear presence of field coccidia resistant to chemical measures, in which a high percentage of *E. acervulina* and *E. maxima*, and a very high percentage of *E. tenella* isolates were shown to have partial or full resistance to a nicarbazin and narasin mixture (Bafundo et al., 2008). This drug combination has been used within the poultry industry for a considerable length of time (Shivaramaiah et al., 2014). The existence of drug resistance to this combination illustrates the growing problem of coccidial drug resistance as a whole.

Vaccines

Vaccination against coccidiosis is one alternative to chemical use. When vaccinating against coccidiosis, the natural immune system of the animal is employed to combat potential infections in the future (Shivaramaiah et al., 2014). Conventionally, live or attenuated parasites are utilized and *Eimeria* specific vaccines can incorporate multiple species or strains (Shivaramaiah et al., 2014). Typical means of attenuation include “irradiation, chemical treatments,” passage through a species host, or a combination of such methods (Shivaramaiah et al., 2014). According to Shivaramaiah and coworkers (2014), attenuated *Eimeria* parasites can be selected through “precociousness,” in which “drug-sensitive, virulent strains of *Eimeria* spp.” are allowed to pass through a species host, reproducing, and being developed into attenuated vaccines (Shivaramaiah et al., 2014). The use of vaccines commercially, however, can be rather difficult due to

production costs and administration. Common methods of large-scale administration include dosing via feed or water, spray vaccine, or gel pucks. However, if the vaccine uptake is not adequate, then "nonuniform immunization" could occur, causing uneven protection (Shivaramaiah et al., 2014).

Chemical Alternatives

As Scheurer et al. (2013) illustrates, outbreaks of coccidiosis can severely affect broiler production due to tissue damage in the intestinal tract. This in turn can "disturb … digestive processes and nutrient absorption, leading to dehydration, blood loss, poor skin pigmentation, and increase susceptibility to other diseases" (Scheurer et al., 2013). Current methods of controlling coccidiosis depend on "managerial skills and the use of prophylactic coccidiostat drugs" (Tewari and Maharana, 2011). However, the poultry industry is now experiencing increasing drug resistance in *Eimeria* strains (Abbas et al., 2012). Thus, pressure from decreasing chemical efficacy has increased demand for new treatment methods such as through plant products. As there are clear advantages to an effective controlling agent without complications with *Eimeria* drug resistance, there is merit in searching for effective methods with mechanisms alternative to traditional anticoccidial chemotherapeutics (Naidoo et al., 2008, as cited in Masood et al., 2013). Attention is being directed towards medicinal plants, plant extracts, and essential oils to potentially combat coccidiosis as "some plants … have the potential to alleviate coccidiosis and reduce its severity" (Bozkurt et al., 2014). According to Muthamilselvan et al. (2016), the use of medicinal plants to combat coccidiosis has potential, as they "contain multiple phytochemicals and can intervene in multiple disease related signaling pathways." Some of these plant varieties have shown success in displaying anticoccidial action by disrupting

developmental stages in the life cycle of *Eimeria* (Muthamilselvan et al., 2016). Plants such as cumin that are shown to have antioxidant effects and artemisinin that creates oxidative stress, "reduce the severity of coccidial infections" (Orengo et al., 2012; Allen et al., 1997; Allen et al., 1998). Artemisinin had been identified as an antimalaria agent derived from *Artemisia annua*. When fed to birds experimentally at 5%, artemisinin afforded significant lesion protection against *E. tenella*, although not *E. maxima* or *E. acervulina* (Allen et al. 1997).

Essential oils

Essential oils (EOs) are "aromatic oily liquids" that are comprised mainly of "cyclic hydrocarbons (monoterpenes) and their alcohol, aldehyde or ester derivatives" (Wallace et al., 2010, as cited in Idris et al., 2017). According to Reisinger et al., (2011), EO's are know to induce multiple differents effects in poultry, "including growth promotion and modulation of the immune system" and therefore have potential to act as a treatment against coccidiosis (Reisinger, et al., 2011). The general mechanisms of EOs involves inhibiting biochemical processes by hindering the cationic transfer of hydrogen and potassium across the cellular membrane (Idris et al., 2017). Although the use of EOs at present to treat coccidiosis may not be entirely viable, when combined with vaccinations or anticoccidial drugs could potentially be a feasable strategy (Idris et al., 2017).

Saponins

Saponins are one such plant product that show promise in demonstrating coccidiosis inhibition. Saponins are a natural plant detergent that are capable of binding to protozoan membrane cholesterol, which leads to "eventual cell lysis and cell death" (Francis et al., 2002, as cited in

Oelshlager, 2018). This binding is possible because the synthesis of cholesterol and saponins "proceed through a common synthetic pathway" (Hassan S. M., 2008). The use of saponins derived from medicinal plants shows promise as they have been observed to "improve nutrient digestibility, growth performance, and odor control" (Oelschlager M., 2018). Saponins derived from a dietary supplementation of the guar bean "suppressed coccidiosis in chickens" (Muthamilselvan et al., 2016, as cited in Hassan et al., 2008). Hassan et al., (2008) predicts that the suppression is due to the active compounds binding to "sterol molecules present on the cell membrane" in *E. tenella*. In further studies, "saponins were presumed to be the active compounds which could lyse oocysts" (Muthamilselvan et al., 2016, as cited in Hassan et al., 2008). Overall, over 1200 plants have been reported to have anti-protazoal properties, but only 20 have been studied thus far for treatment of coccidiosis. (Yang et al., 2019). Thus, there is an immense number of unexplored options for coccidiosis therapies alternative to commonly used drugs.

Chapter III. METHODS AND MATERIALS

Challenge strains

Oocysts of *Eimeria maxima* M6 (EM) and wild-type *Emeria acervulina* (EA) were provided by Dr. John. R. Barta, University of Guelph, Canada. The methods for detecting and recovering oocysts from infected chickens, oocyst sporulation, and the preparation of infective doses, were conducted as described previously (Haug et al., 2006). A dose titration study was performed to determine the EM/EA coccidia co-challenge dose before starting the experimental trial. At 13 days of age, broilers were weighed, divided into three groups (n = 15/group), and challenged with three different doses (10,000, 20,000, or 40,000) of sporulated oocysts in 1 mL volume by oral gavage. The fourth group of chicks was kept as a negative control. Five days post-challenge, body weight (BW) and body weight gain (BWG) were recorded. In the present study, challenged chickens were orally gavaged at 9:00 am with the mixed culture of EM/EA (10,000 sporulated EM containing 4% wild-type EA) at 14 days of age as this dosage reduced BWG by 35.82%. This is based on the criterion that the challenge dose must cause sub-clinical coccidiosis, consisting of a reduction between 25-35 % in BWG without the presence of clinical signs.

Animal source and experimental design

Two hundred and forty-one-day-old male Cobb-Vantress broiler chickens (Fayetteville, AR, USA) were weighed and randomly allocated to one of three groups with ten replicates (n=8 chickens/replicate). Chickens were placed in battery cages, with a controlled age-appropriate environment: Group 1) Negative control (no challenge or treatment); 2) Challenge control (*Eimeria* challenge only); 3) Challenge + Salinomycin at 60 g/ton (Bio-Cox 60,

Huvepharma, Peachtree City, GA 30269). Chicks received *ad libitum* access to water and feed for 23 days. An experimental starter diet (Table 1) was formulated to approximate the nutritional requirements of broiler chickens as recommended by the National Research Council (NRC, 1994) and adjusted to breeder's recommendations (Cobb, 2015). Chickens received 23 hours of light from days 1 to 4, 20 hours of light from days 5 to 14, and 18 hours of light from days 15 to 23. Light intensity was set at 30- footcandle the first week, 1-foot candle from days eight to fourteen, and 0.5-footcandle from days 15 to 23. Temperature and light were set to mimic commercial conditions from day 1-21 in all rooms with a gradual reduction on temperature from 32 to 24°C and relative humidity at 55 ± 5%. Performance parameters: body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion rate (FCR) were recorded at days 7, 14, 20, and 23. On day 20, half of the chickens from each replicate were weighed and euthanized while the remaining chickens were weighed and euthanized on day 23 in order to evaluate macroscopic lesions according to the scoring system of Johnson and Reid (Johnson and Reid, 1970). Oocyst per gram (OPG) was evaluated on days six, seven, and eight post-challenge, and samples were collected at 9:00 AM and 6:00 PM on each day, respectively. All animal handling procedures complied with the Institutional Animal Care and Use Committee (IACUC) at the University of Arkansas, Fayetteville. Explicitly, the IACUC approved this study under protocol #21020.

Feed weight

During the trial, birds received *ad libitum* access to feed and water. The total feed consumed was recorded for each cage at the end of the trial. Each cage had a respective bucket to store/ transport feed in. Feed was weighed inside the bucket and recorded. All feed placed into pan and turbo

feeders was removed from the cage's corresponding bucket at 7 days of age. Any feed that was discarded or added into the buckets was weighed and recorded. At the end of the trial, all remaining feed in the buckets and feeders was weighed and subtracted from the initial weight to calculate the total feed consumed. The weight of the bucket and feeders was accounted for and subtracted from the total weight during calculations.

Feed intake calculation

The total feed intake per cage was done by weighting the remaining feed and subtracted from the weight of the initial feed (10,000 grams), then divided by the number of birds present in the cage to obtain the average.

Feed remaining = $Total\ weight - (Bucket\ weight + Feeder\ weight)$

Total feed intake = $10{,}000\ grams - Feed\ remaining$

Average feed intake = $\frac{Total\ feed\ intake}{n\ birds}$

Data and statistical analysis

Lesions scores, oocyst per gram, and performance data were subjected to ANOVA as a completely randomized design using the GLM procedure of SAS (SAS, 2002). For growth performance parameters (BW, BWG, FI, and FCR), each replicate cage was considered as an experimental unit. Treatment means were partitioned using Duncan's multiple range test at $P<0.05$ indicating statistical significance.

Results and discussion

The results of the evaluation of body weight, body weight gain, feed intake, and feed conversion ratio in broiler chickens challenged with coccidia are summarized in Table 2. All three groups started with similar BW; however, at day 7, there was an increase in the BW of chickens treated with Salinomycin. By day 20 (6 days post-challenge), the negative control group (no challenged or treated) and challenge Salinomycin treated group exhibited a significant increase in BW when compared with the challenged control group ($P < 0.05$). Interestingly, by day 23 (9 days post-challenge), there were only significant differences in BW between the negative control group and challenge control. A similar trend was observed in BWG and FCR. No significant differences were observed in FI among the three groups (Table 2).

Table 3 shows the results of the evaluation of *E. maxima* oocyst per gram count in the feces of broilers on day 6 through day 8 post-challenge at different times of the day, the average per day. Although there was some recovery of oocysts from unchallenged untreated control chickens, there was significantly less OPG in this group compared to both challenge groups. No significant difference in OPG was observed between both challenge groups during the three days of evaluation (Table 3). In the present study, it was remarkable to find that EM oocysts were excreted in very high numbers on day 6 post-challenge in the evening for all three experimental groups. When combining and obtaining the average OPG, day 6 showed a higher number of EM oocysts, and the expected significant differences between the OPG amongst the three experimental groups were observed (Table 3). Similarly, significant differences were found in the lesion

scores for EM for both evaluation days (20 and d 23) between the three experimental groups. Nevertheless, a higher number of oocysts were recovered on d 20 in both challenged groups compared to d 23 (Table 3). In the present study, negative control chickens were randomly assigned into the experimental groups that were challenged with coccidia. Perhaps, that is the reason that these chickens showed some infection, due to cross contamination of feces among the cages. Clearly, in future studies, negative control chickens must be placed in a separate room and if this is not possible, separate, and isolated cages.

The results of the evaluation of *E. acervulina* oocyst per gram count in the feces of broilers on day 6 through day 8 post-challenge at different times of the day, the average per day, and lesions scores on days 20 and 23 are summarized on Table 4. A similar trend was observed in the OPG for EA, although more oocysts were present on day 23 in the challenged groups than day 20 (Table 4). Macroscopic intestinal lesions scores on days 20 and 23 of age are showed in table 5. In summary, oocyst counts were significantly different between morning and evening on d 6 post-challenge. The increased shedding of oocysts in the evening samples collections are in accordance with earlier studies of the diurnal excretion of *Eimeria* spp. oocysts (Hudman et al., 2000; Brown et al., 2001; Misof, 2004).

Coccidiosis remains one of the most critical diseases in poultry and results in the annual loss of millions of US dollars by the poultry industry (Williams, 2005; Chapman, 1999). One common practice in managing coccidiosis is the use of prophylactic drugs and

antimicrobials that inhibit the development of sporozoites/ merozoites. However, the poultry industry is now experiencing increasing drug resistance in *Eimeria* strains (Abbas et al., 2012). Thus, pressure from decreasing chemical efficacy has increased demand for new treatment methods such as through plant products. As there are clear advantages to an effective controlling agent without complications with *Eimeria* drug resistance, there is merit in searching for effective methods with mechanisms alternative to traditional anticoccidial chemotherapeutics (Naidoo et al., 2008; Masood et al., 2013). Vaccination against coccidiosis is one alternative to chemical use. When vaccinating against coccidiosis, the natural immune system of the animal is employed to combat potential infections in the future (Shivaramaiah et al., 2014). Conventionally, live or attenuated parasites are utilized and *Eimeria* specific vaccines can incorporate multiple species or strains (Shivaramaiah et al., 2014). Attenuated *Eimeria* parasites can be selected through "precociousness," in which "drug-sensitive, virulent strains of *Eimeria* spp." are allowed to pass through a species host, reproducing, and being developed into attenuated vaccines (Peek and Landman, 2011; Shirley et al., 2007).

Previous research has described circadian variation in oocyst shedding across multiple avian host species (Hudman et al., 2000; Brown et al., 2001). Consequently, if circadian variation in oocyst shedding is not accounted for, the results of such testing are unreliable and may be misleading (Misof, 2004). A suitable method for obtaining accurate data seems to be to restrict the sampling period.

The results of this study show that the day and the time at which samples are collected

can have a significant impact on data and reinforces the importance of collecting the fecal samples at the same time of day post-challenge. Oocyst counts were significantly different between morning and afternoon on day six post coccidia challenge. Coccidia load sampling should be restricted to the second half of the total daylight time. This more restrictive period should thus be considered as the preferred period for obtaining reliable information.

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Table 1. Ingredient composition and nutrient content of a corn-soybean starter diet used in all experimental groups on as-is basis.

Item	Starter diet
Ingredients (%)	
Corn	57.34
Soybean meal	34.66
Poultry fat	3.45
Dicalcium phosphate	1.86
Calcium carbonate	0.99
Salt	0.38
DL-Methionine	0.33
L-Lysine HCl	0.31
Threonine	0.16
Vitamin premix[1]	0.20
Mineral premix[2]	0.10
Choline chloride 60%	0.20
Antioxidant[3]	0.02
Calculated analysis	
Metabolizable energy (kcal/ kg)	3,035
Crude protein (%)	22.16
Ether extract (%)	5.68
Lysine (%)	1.35
Methionine (%)	0.64
Methionine + cystine (%)	0.99
Threonine (%)	0.92
Tryptophan (%)	0.28
Total calcium	0.90
Available phosphorus	0.45
Determined analysis	
Crude protein (%)	21.15
Ether extract (%)	6.05
Calcium (%)	0.94
Phosphorus (%)	0.73

[1]Vitamin premix was supplied by the following per kg: vitamin A, 20,000 IU; vitamin D3, 6,000 IU; vitamin E, 75 IU; vitamin K3, 6.0 mg; thiamine, 3.0 mg; riboflavin, 8.0 mg; pantothenic acid, 18 mg; niacin, 60 mg; pyridoxine, 5 mg; folic acid, 2 mg; biotin, 0.2 mg; cyanocobalamin, 16 µg; and ascorbic acid, 200 mg (Nutra Blend LLC, Neosho, MO 64850). [2]Mineral was premix supplied at the following per kg: manganese, 120 mg; zinc, 100 mg; iron, 120 mg; copper, 10 to 15 mg; iodine, 0.7 mg; selenium, 0.4 mg; and cobalt, 0.2 mg (Nutra Blend LLC, Neosho, MO 64850). [3]Ethoxyquin.

Table 2. Evaluation of body weight, body weight gain, feed intake, and feed conversion ratio in broiler chickens challenged with coccidia.

Item	Negative control (no challenge or treatment)	Challenge control (*Eimeria* challenge only)	Challenge + treated Salinomycin Sodium (60 g/ton)
Body weight (g)			
d 0	46.60 ± 0.19	46.16 ± 0.36	46.70 ± 0.42
d 7	145.05 ± 2.16 ^{ba}	144.48 ± 2.51 ^{ba}	148.95 ± 2.33 ^{a}
d 14	401.30 ± 7.28 ^{bc}	402.63 ± 9.21 ^{bac}	429.16 ± 5.11 ^{a}
d 20	736.14 ± 11.23 ^{a}	671.97 ± 16.35 ^{b}	751.09 ± 8.00 ^{a}
d 23	927.51 ± 20.06 ^{a}	781.25 ± 42.28 ^{b}	881.38 ± 23.87 ^{ba}
Body weight gain (g)			
d 0 to 7	98.45 ± 2.13 ^{ba}	98.31 ± 2.54 ^{ba}	102.25 ± 2.36 ^{a}
d 7 to 14	256.26 ± 6.15 ^{bc}	258.16 ± 7.55 ^{bc}	280.21 ± 3.82 ^{a}
d 14 to 20	334.84 ± 5.67 ^{a}	269.34 ± 11.69 ^{b}	321.93 ± 5.47 ^{a}
d 0 to 23	890.31 ± 29.18 ^{a}	735.65 ± 42.60 ^{b}	834.03 ± 23.61 ^{ba}
Feed intake (g)			
d 0 to 14	609.89 ± 12.51 ^{a}	621.93 ± 16.27 ^{a}	641.06 ± 15.45 ^{a}
d 0 to 20	930.19 ± 37.87 ^{a}	909.81 ± 36.98 ^{a}	770.41 ± 30.53 ^{a}
d 0 to 23	1323.79 ± 31.08 ^{a}	1198.68 ± 57.19 ^{a}	1325.55 ± 29.11 ^{a}
Feed conversion ratio (adjusted)			
d 0 to 14	1.50 ± 0.03 ^{a}	1.53 ± 0.03 ^{a}	1.50 ± 0.04 ^{a}
d 0 to 20	1.44 ± 0.01 ^{b}	1.49 ± 0.02 ^{ba}	1.45 ± 0.02 ^{b}
d 0 to 23	1.41 ± 0.06 ^{b}	1.54 ± 0.03 ^{ba}	1.51 ± 0.03 ^{ba}

Chickens were challenged with *Eimeria maxima* (M6) and *Eimeria acervulina* (wild type) by oral gavage at 14 days. $^{a-c}$ Mean values in the same row that do not share a common letter differ significantly ($P < 0.05$). Each value represents the mean ± standard error. Ten replicate pens of each treatment with n = 8 chicks per pen were used.

Table 3. Evaluation of *E. maxima* oocyst per gram count[1] in the feces of broilers on day 6 through day 8 post challenge at different times of the day and average per day.

Treatment	Day 6 / 9:00 AM (day 20)	Day 6 / 6:00 PM (day 20)	Day 6 Average AM/PM	Day 7 / 9:00 AM (day 21)	Day 7 / 6:00 PM (day 21)	Day 7 Average AM/PM	Day 8 / 9:00 AM (day 22)	Day 8 / 6:00 PM (day 22)	Day 8 Average AM/PM
Negative control	239 ± 123.12 ^{bz}	1,880 ± 350.09 ^{by}	1,059 ± 192.37 ^{bz}	2,214 ± 2,055.28 ^{by}	465 ± 164.91 ^{bz}	1,340 ± 1,080.81 ^{bz}	312 ± 247.92 ^{bz}	504 ± 399.77 ^{bz}	408 ± 319.28 ^{by}
Challenge control	39,756 ± 8,540.64 ^{ay}	392,859 ± 53742.38 ^{ay}	216,308± 26,680.84 ^{ax}	258,783 ± 34093.03 ^{aw}	73,803 ± 20,753.83 ^{ax}	166,293 ± 24,541.92 ^{ay}	39,751 ± 10,808.39 ^{ay}	12,844 ± 2,256.07 ^{az}	26,298 ± 5888.63 ^{az}
Challenge + treated	28,060 ± 11,708.46 ^{ay}	304,517 ± 31,024.37 ^{ay}	166,288 ± 11,708.46 ^{ay}	180,752 ± 39,771.21^{aw}	86,940 ± 22,231.97 ^{ax}	133,846 ± 39,771.21 ^{ay}	23,440 ± 5,199.72 ^{ayz}	11,966 ± 1,207.11 ^{az}	17,703 ± 5199.72 ^{az}

Table 4. Evaluation of *E. acervulina* oocyst per gram count[1] in the feces of broilers on day 6 through day 8 post challenge at different times of the day and average per day.

Treatment	Day 6 / 9:00 AM (day 20)	Day 6 / 6:00 PM (day 20)	Day 6 Average AM/PM	Day 7 / 9:00 AM (day 21)	Day 7 / 6:00 PM (day 21)	Day 7 Average AM/PM	Day 8 / 9:00 AM (day 22)	Day 8 / 6:00 PM (day 22)	Day 8 Average AM/PM
Negative control	83 ± 28.07 ^{ay}	52 ± 30.20 ^{cy}	55 ± 29.65^{cy}	0 ± 0^{cz}	0 ± 0 ^{bz}	0 ± 0 ^{cz}	52 ± 52.08 ^{cy}	26 ± 26.25 ^{cy}	52 ± 52.21 ^{cy}
Challenge control	1,000 ± 319.03 ^{ayz}	5,993 ± 995.74 ^{aw}	3,497 ± 352.58 ^{ay}	6,656 ± 1924.02 ^{aw}	3,007 ± 1266.66 ^{ax}	4,831 ± 1580.37 ^{ay}	1,800 ± 212.01 ^{aby}	779 ± 228.56 ^{abz}	1,289 ± 182.52^{baz}
Challenge + treated	194 ± 54.9 ^{az}	1,849 ± 498.62 ^{bw}	1,022 ± 241.84 ^{by}	2,287 ± 335.90 ^{bw}	1,912 ± 514.33 ^{aw}	2,099 ± 270.65 ^{by}	1,339 ± 88.24 ^{bx}	552 ± 99.01 ^{by}	945 ± 69.81^{bz}

Chickens were challenged with *Eimeria maxima* (M6) and *Eimeria acervulina* (wild type) by oral gavage at 14 days of age. [1]Each value represents the mean ± standard error. Five replicates/ n = 5. $^{a-c}$ Mean values in the same column that do not share a common letter differ significantly. $^{w-z}$ Mean values in the same row that do not share a common letter differ significantly ($P < 0.05$). [2]Each value represents the mean ± standard error. Ten replicate pens of each treatment with n = 8 chicks per pen were used. $^{a-c}$ Mean values in the same column that do not share a common letter differ significantly.

Table 5. Macroscopic intestinal lesions scores[1] on days 20 and 23 of age for broiler chickens challenged with *Eimeria acervulina* and *E. maxima*.

Treatment	*Eimeria acervulina*		*Eimeria maxima*	
	day 20	day 23	day 20	day 23
Negative control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.05 ± 0.03 ^{c}	0.00 ± 0.00 ^{c}
Challenge control	0.80 ± 0.12$^{a\,z}$	1.45 ± 0.11$^{a\,y}$	1.93 ± 0.10 $^{a\,y}$	1.25 ± 0.08 $^{a\,z}$
Challenge + treated Salinomycin Sodium	0.48 ± 0.11$^{b\,z}$	0.90 ± 0.09$^{b\,y}$	1.28 ± 0.14 $^{b\,y}$	0.77 ± 0.09 $^{b\,z}$

Chickens were challenged with *Eimeria maxima* (M6) and *Eimeria acervulina* (wild type) by oral gavage at 14 days of age. [1]Each value represents the mean ± standard error. Five replicates, n = 5. $^{a\text{-}c}$ Mean values in the same column that do not share a common letter differ significantly. $^{w\text{-}z}$ Mean values in the same row that do not share a common letter differ significantly, ($P < 0.05$). [2]Each value represents the mean ± standard error. Ten replicate pens of each treatment with n = 8 chicks per pen were used. $^{a\text{-}c}$ Mean values in the same column that do not share a common letter differ significantly, ($P < 0.05$).

1. Figure 1: Table 1. Ingredient composition and nutrient content of a corn-soybean starter diet used in all experimental groups on as-is basis.

2. Figure 2: Table 2. Evaluation of body weight, body weight gain, feed intake, and feed conversion ratio in broiler chickens challenged with coccidia.

3. Figure 3: Table 3. Evaluation of *E. maxima* oocyst per gram count in the feces of broilers on day 6 through day 8 post challenge at different times of the day and average per day.

4. Figure 4: Table 4. Evaluation of *E. acervulina* oocyst per gram in the feces of broilers on day 6 through day 8 post challenge at different times of the day and average per day.

5. Figure 5: Table 5. Macroscopic intestinal lesions scores on days 20 and 23 of age for broiler chickens challenged with *Eimeria acervulina* and *E. maxima*.