

Research Note: Evaluating fecal shedding of oocysts in relation to body weight gain and lesion scores during *Eimeria* infection

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ABSTRACT Coccidiosis has been a pervasive disease within the poultry industry, with test parameters used to measure effectiveness of treatment strategies often being subjective or influenced by non-disease-related activity. Four experiments were completed, which examined several test parameters of coccidiosis, including body weight gain (BWG), lesion scores, and oocysts per gram of feces (OPG). Each experiment included at least 2 parameters for measuring coccidial infection in chickens and turkeys. In experiment 1, an inoculated control was measured against 3 anticoccidial groups, whereas in experiments 2 to 4, noninoculated and inoculated controls were compared via BWG and OPG. Lesion scores were also included in experiments 1,

3, and 4. Experiment 4 resulted in high correlation, via Pearson correlation coefficient, between BWG and OPG ($r = -0.69$), very high correlation between OPG and lesion score ($r = 0.86$), and moderate correlation between BWG and lesion score ($r = -0.49$). Lesion scores proved to be effective in confirming *Eimeria* infection, although they did not correlate well with BWG or OPG. Each parameter tended to provide more useful information when lined up with the *Eimeria* life cycle. Incorporation of OPG, with BWG and lesion scores, as test parameters to measure coccidiosis intervention strategies, provides a global description of disease that may not otherwise be observed with the 2 latter measurements alone.

Key words: *Eimeria*, coccidiosis, oocysts per gram of feces, fecal shedding

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INTRODUCTION

Coccidiosis, caused by protozoan parasites of the apicomplexan genus *Eimeria*, has been recognized as an issue within the poultry industry for over 90 yr (Chapman, 2014), with economic impacts estimated upward of \$3 billion worldwide (Williams, 1999; Dalloul and Lillehoj, 2006). Infections in chickens and turkeys are species specific and are commonly identified by the region of gastrointestinal tract (GIT) in which they infect (Chapman, 2008; Chapman et al., 2013). Owing to the strong survival rate of oocysts within the environment, *Eimeria* is ubiquitous, and strong management programs, both prophylactic and therapeutic, are essential for control of the disease (Chapman et al., 2013; Price et al., 2013).

Recognized as a serious issue in the poultry industry, a variety methods to both treat and control coccidial infection, including ionophores, have been commonly used to effectively limit the impacts of coccidiosis (Smith et al., 1981; Long and Jeffers, 1982; Mehlhorn et al., 1983). Even though these treatment strategies have been historically effective, limitations on the use of antibiotics through the Veterinary Feed Directive and consumer demand on the poultry industry have pressured producers to seek other options (Veterinary Feed Directive, 2015). Another common control method has been to inoculate birds with low levels of live *Eimeria* to induce immunity (Shirley, 1989). Although generally successful, live coccidiosis vaccination relies on proper *Eimeria* cycling through each flock and is management intensive, and cross-protection to wild-type strains is not 100% effective (Joyner, 1969; Martin et al., 1997; Williams, 2002). To limit the ubiquity of the parasite, effective alternative treatment and prevention strategies should continue to be developed.

While developing these strategies, it is important to consider ideal factors by which to evaluate efficacy. Test parameters that have been widely utilized in research include body weight gain (BWG), feed

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conversion ratio, macroscopic lesion score (**LS**), and to a lesser extent, fecal shedding of *Eimeria* oocysts, typically measured as oocysts per gram of feces (**OPG**). Growth performance parameters, such as BWG and feed conversion ratio, may be influenced by other factors beyond coccidial infection, occasionally making interpretation challenging (Allen and Fetterer, 2002; De Gussem, 2007; Chapman et al., 2013). Alternatively, LS provide infection confirmation and gross damage, but scoring is subjective and only captures a specific time point, which does not quantify pathogen load. Given the drawbacks of each of these methodologies, it is important to incorporate other measurements of disease that can capture physiologic effects that may otherwise be missed. Quantification of OPG provides information at the infection level and reproduction of *Eimeria* within the GIT, which may be used to monitor vaccine response within a flock, and demonstrate treatment impact on overall *Eimeria* life cycle (Braunius, 1985). The incorporation of OPG along with BWG and LS may provide another tool for determining the effectiveness of intervention strategies. The experiments presented here evaluated OPG as a method of measuring prophylactic anticoccidial treatments in relation to LS and BWG to provide insight into how it may be helpful as a standard tool for evaluating efficacy of intervention strategies.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

A total of 4 experiments were completed, in which either day of hatch Ross 708 broilers or commercial cross turkeys were obtained from a local hatchery, neck-tagged, and randomly placed into floor pens with fresh pine shaving litter. During the first week, temperature was maintained at 35°C with 24 h light, followed by age-appropriate ambient temperature and gradual reduction in lighting after 1 wk to a 20:4 h light:dark schedule. Nutritionally complete unmedicated feed and water were provided *ad libitum*. All animal handling protocols were in compliance with Institutional Animal Care and Use Committee requirements at The Ohio State University.

***Eimeria* spp. Preparation**

For all experiments, *Eimeria* were prepared and administered to inoculated groups using purified oocyst cultures diluted in 0.9% saline as described by Wilson et al. (2018). Experiments 1 and 3 included an *Eimeria maxima* (**EMax**) Guelph strain inoculation, whereas experiment 2 involved hatchery vaccination with *Eimeria acervulina* (**EAcerv**), EMax, and *Eimeria tenella* (**ET**). The turkey experiment included a mixed dose of *Eimeria adenoeides* (**EAd**) and *Eimeria meleagridis* (**EMel**).

Experiment 1

A total of 90 day-of-hatch broiler chicks were randomly assigned to one of four treatment groups that consisted of a challenged control (**CC**), or 3 anticoccidial groups (**AC 1-3**). The specifics of each group have not been further described because the particular treatments were not relevant to evaluation of OPG data as a method of measuring treatment impact. On day 23, 10,000 oocysts/bird of EMax was administered to all groups via oral gavage. Body weight was measured on day 23 and day 28, following the disease period. On day 28, 50% of the birds were euthanized for macroscopic LS evaluation using the method established by Johnson and Reid (1970). The remaining birds were moved to wire floor cages at 3 cages per treatment for total fecal collection to evaluate fecal shedding of oocysts, calculated as OPG, on day 29, which was 6 D post infection (**DPI**). Fecal samples were collected in bags, weighed, and suspended in a 3-fold dilution of 2% PDC (Sigma-Aldrich, Co., 3050 Spruce Street, St. Louis, MO, USA), then quantified as described by Hodgson (1970).

Experiment 2

A total of 900 day-of-hatch broiler chicks were randomly assigned to either a nonchallenged (**NC**) or CC group, at 50 birds per pen, and 9 replicate pens per treatment. The CC group received a three-way coccidia vaccine on day of hatch at the hatchery. Individual or pen body weight was measured on day 0, day 7, day 14, day 21, and day 35. Approximately 10 to 12 fresh fecal droppings were collected from each pen and combined into one sample per pen at day 10, day 17, and day 24 to calculate OPG, via methods described earlier.

Experiment 3

A total of 160 day-of-hatch broiler chicks were randomly assigned to either NC or CC, with 20 birds per pen, and 4 replicate pens per treatment. On day 14, EMax was orally administered at 20,000 oocysts/bird via oral gavage to the CC group. Body weights were measured on day 14, day 19, and day 28, with LS evaluated on day 19 and day 28 from 5 birds per pen, for a total of 20 birds per treatment. Multiple fresh feces were collected from each pen to form an individual sample per pen on day 19 to 23 to monitor oocyst shedding, and calculate OPG at peak shedding, as described earlier, on day 21, 7 DPI.

Experiment 4

A total of 360 day-of-hatch turkey poults were randomly assigned to either NC or CC, with 30 birds per pen, and 6 pens per treatment. On day 16 of the experiment, turkeys in CC were orally inoculated with 6,250 EAd and 25,000 EMel oocysts each. Body weights were measured on day 0, day 16, day 21, and day 28 to determine BWG. Five birds per pen were euthanized

to determine LS on day 21 and day 28. Beginning on day 21, 10 to 12 fresh fecal droppings were collected from each pen and pooled to create a single sample once per day on day 21 day 22 and day 25–26 to calculate OPG shedding peaks, as described earlier.

Statistical Analysis

Birds were the experimental unit for BWG and LS, whereas pens were the experimental unit for OPG and pen BWG. The BWG and OPG data were subject to analysis of variance as a completely randomized design using the General Linear Models procedure of SAS (JMP software, SAS Inc., Cary, NC; 2016). *Eimeria* LS were subject to analysis of variance as a completely randomized design using the Proc Mixed procedure of SAS (SAS 6.4 software, SAS Inc.; 2016). Significant differences among the means for LS in experiments 1, 3, and 4 were determined using Tukey HSD test at $P < 0.05$. Significant differences among BWG and OPG means were determined by using Student t-test in all 4 experiments at $P < 0.05$. All values are expressed as mean \pm SE. Correlations between BWG, LS, and OPG data were determined using Pearson correlation coefficient for all 4 experiments, represented as very low (0.000–0.249), low (0.250–0.449), moderate (0.450–0.649), high (0.650–0.849), very high (0.850–1.000).

RESULTS AND DISCUSSION

Eimeria infection has been shown to result in decreased feed efficiency that can be attributed to disruption of the intestine caused by *Eimeria* during the endogenous portion of its life cycle (Sharman et al., 2010). Coccidial infection has been linked to reduced growth performance parameters, decreased feed and water intake, altered intestinal pH, decreased viscosity of the digesta, and malabsorption of nutrients (Williams, 2005). Several factors, such as *Eimeria* species and strain, pathogen load, and site of infection, affect disease severity of coccidial infection and have a varying range of impacts on overall bird health (Williams, 2005; Chapman, 2014). For these experiments, EMax was selected because it has been one of the most commonly diagnosed coccidial species worldwide (Schnitzler and Shirley, 1999), whereas EAcer, ET, EAd, and EMel were selected because of their common inclusion in live vaccines (Williams, 2002).

The first experiment evaluated BWG, LS, and OPG at 6 DPI to determine the impact of 3 different treatments against EMax. Only the relationship of each of these parameters at determining level of *Eimeria*-related morbidity was evaluated, rather than the effect of treatment, which was used to determine the best components to reflect level of disease. The range of mean LS was 1.30 to 2.00, which indicated a moderate disease level, and although there was no statistical difference ($P < 0.05$) among treatments, AC3 had the highest LS with AC2 at the lowest, and CC in the middle of the range (Table 1). However, BWG showed CC with the highest

mean BWG at 404.55 ± 19.69 g/bird, followed by AC 1, 2, and 3 at 388.35 ± 11.43 , 367.55 ± 28.00 , and 327.46 ± 15.42 , respectively, with AC3 having a significantly lower BWG ($P = 0.006$) than the remaining 3 groups. A low negative correlation was observed, determined by Pearson correlation coefficient, between BWG and LS ($r = -0.43$), which suggested that even with a moderate challenge, BWG and LS did not provide consistent, or matching, descriptions of disease impact (Table 1). However, the pattern of results for OPG at 6 DPI, the day of peak shedding for EMax, was similar to that of BWG (Table 1). In this case, CC had the lowest ($P = 0.025$) detected OPG at 183.67×10^3 , followed by AC 1, 2, and 3 at 306.00×10^3 , 392.00×10^3 , and 482.33×10^3 , respectively. By including oocyst shedding as a measurement of morbidity, the ranking of treatment efficacy became more obvious because it was similar to BWG, but with only BWG and LS, it would have been difficult to draw conclusions.

Although experiment 2 did not evaluate LS, both BWG and OPG were monitored after *Eimeria* vaccination on day of hatch. Although fecal samples were collected as a means to monitor cycling of oocysts after vaccination, they could also be compared to BWG data to infer how well the 2 different parameters conveyed *Eimeria* activity and pathogenesis in treatment groups. Pen BWG was measured from day 7–14, day 14–21, and day 21–35, with no statistical differences observed between NC and CC at any period (Table 2). At day 10 OPG sampling, no differences in shedding were observed, possibly owing to low levels of *Eimeria* in both NC and CC at only $0.04 \times 10^3 \pm 0.03 \times 10^3$ OPG and $2.06 \times 10^3 \pm 0.91 \times 10^3$ OPG, respectively (Table 2). This suggested low-level oocyst contamination of NC, likely via cross contamination at the hatchery because birds were placed on fresh litter. On day 17 and day 24, OPG reflected a statistical difference ($P = 0.039$, $P = 0.020$, respectively) between NC and CC, whereas pen BWG showed no differences on day 14–21 or day 21–35 (Table 2). Very low correlations were observed between pen BWG day 7–14 and OPG day 10 ($r = -0.24$) and between pen BWG day 14–21 and OPG day 17 ($r = 0.04$), and a low correlation was observed between day 21–35 pen BWG and OPG day 24 ($r = 0.26$, Table 2). This would suggest that, perhaps, OPG and BWG were not good options for co-monitoring of disease with these time points. Considering peak oocyst shedding typically occurs around 5 to 7 DPI (Conway et al., 1999; Allen and Fetterer, 2002; Al-Badri and Barta, 2012), impact of the *Eimeria* life cycle relative to OPG, LS, and BWG should be taken into account when comparing these parameters. However, without any OPG monitoring, no clear explanation for BWG similarity between NC and CC would be available. This highlighted the importance of tracking oocyst shedding. There has been evidence that BWG is sometimes not effected, even when oocysts have been ingested (Zhu et al., 2000; Yim et al., 2011; Barrios et al., 2017). These findings further emphasized

Table 1. Body weight gain, lesion score, and peak *Eimeria* shedding of broilers after coccidial infection, experiment 1.¹

Group	Day 23–28 BWG (g)	Day 28 LS	Day 29 OPG (6 DPI), ×10 ³
CC	404.55 ± 19.69 ^a	1.73 ± 0.24	183.67 ± 33.20 ^b
AC 1	388.35 ± 11.43 ^a	1.31 ± 0.21	306.00 ± 64.75 ^{a,b}
AC 2	367.55 ± 28.00 ^a	1.30 ± 0.21	392.00 ± 212.71 ^{a,b}
AC 3	327.46 ± 15.42 ^b	2.00 ± 0.31	482.33 ± 54.69 ^a
<i>P</i> -value	0.0059	NS	0.0246
<i>r</i> ²	BWG vs. LS −0.43		

^{a,b}Mean values with different superscript letters within a column indicate a significant difference ($P < 0.05$).

¹All groups received an oral *Eimeria maxima* challenge of 10,000 oocysts/bird on day 23. Challenged control (CC) birds did not receive any anticoccidial treatment, whereas the remaining 3 treatment groups received anticoccidial treatments 1 to 3 (AC 1–3). Body weight was measured in grams per individual bird on day 23 and day 28 to capture the body weight gain (BWG) during the disease period. Lesion scores (LS) were observed and scored on day 28. Total fecal collection was completed daily from birds in wire floor cages, and then enumerated to determine oocysts per gram of feces (OPG). Peak shedding occurred on day 29 at 6 D post infection (DPI). Data presented as mean ± SE.

²Pearson correlation coefficient *r* value.

the need to incorporate other measurements to determine the level of coccidial infection, in addition to performance parameters such as BWG.

When EMax was administered to broilers on day 14 in experiment 3, BWG was not affected by day 19, and low LS were observed, with CC having significantly stronger LS of 0.90 ± 0.14 compared with 0.45 ± 0.11 in NC ($P = 0.033$; Table 3). Both day 20 and day 21 OPG were significantly higher in CC, with 148.10×10^3 , 37.75×10^3 and 375.46×10^3 on 91.42×10^3 OPG detected, respectively, with none detected in NC (Table 3). By day 29, neither BWG nor LS revealed differences between treatment groups. When day 19 LS were compared to day 20 OPG, a high negative correlation was observed ($r = -0.79$), which suggested that as LS decrease, OPG increases. Because LS represent rupture of the enterocytes, whereas OPG represents oocysts shed following rupture, OPG will increase as the intestine heals. A low correlation was observed between pen BWG day 14–19 and day 19 LS ($r = 0.42$), but a very high negative correlation was observed between pen BWG day 14–19 and day 21 OPG at peak EMax shedding ($r = -0.93$, Table 3). The stronger correlation observed by including OPG, in addition to BWG and LS, emphasized the role of OPG in creating a more complete disease description. Infection level differences

detected by OPG monitoring would have been undetectable using only BWG and LS.

The final experiment investigated the impact of EAd and EMel administration at day 16 to turkeys, with BWG, LS, and OPG followed through day 28. The effect of *Eimeria* on BWG was noted within 5 D of challenge, with CC having a lower BWG than NC at 192.52 ± 4.07 g compared with 204.67 ± 3.56 g, respectively ($P < 0.001$; Table 4). Furthermore, although lesions in both the duodenum and ceca were extremely modest at day 21, they were statistically different ($P = 0.039$, $P = 0.027$, respectively; Table 4). The following week, both day 21–28 BWG and day 28 duodenal LS remained different, with CC BWG at 307.26 ± 6.47 g compared with NC at 395.57 ± 4.37 ($P < 0.001$, $P = 0.023$; Table 4). Oocysts detected in feces were reported only for days in which spikes in shedding were measured, because of the synchronized life cycle of *Eimeria* as a result of a singular administration time point, to simplify reporting of results (day 23–24 and day 27–28 data not shown). Throughout the monitoring period, no oocysts were detected in NC, whereas a typical up and down pattern, associated with *Eimeria* oocyst life cycle, was detected in CC (Table 4). High variability between pens likely affected statistical analysis for OPG at day 22 and day 26, but

Table 2. Pen body weight gain and oocysts per gram of feces of broilers after coccidial vaccine on day of hatch, experiment 2.¹

Pen BWG (kg)	Day 7–14	Day 14–21	Day 21–35
NC	8.82 ± 0.15	11.11 ± 0.29	32.95 ± 0.79
CC	8.98 ± 0.12	10.28 ± 0.34	32.31 ± 1.96
<i>P</i> -value	NS		NS
OPG, ×10 ³	Day 10	Day 17	Day 24
NC	0.04 ± 0.03	3.86 ± 2.94 ^b	15.67 ± 5.91 ^b
CC	2.06 ± 0.91	172.71 ± 66.55 ^a	139.75 ± 41.55 ^a
<i>P</i> -value	NS	0.0388	0.0203
<i>r</i> ²	Day 7–14 BWG vs. day 10 OPG	Day 14–21 BWG vs. day 17 OPG	Day 21–35 BWG vs. day 24 OPG
	−0.24	0.04	0.26

^{a,b}Mean values with different superscript letters within a column indicate a significant difference ($P < 0.05$).

¹The nonchallenged (NC) birds did not receive any treatment or challenge. Challenged control (CC) birds received an oral vaccine with a mixture of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* on day of hatch. Body weight gain was measured in grams per individual bird on day 0, day 7, day 21, and day 38, and then totaled per pen, with day 14 measured as pen weight. Fecal samples were collected from each pen and then enumerated to determine oocysts per gram of feces (OPG) at day 10, day 17, and day 24. Data presented as mean ± SE ($n = 9$ pens/treatment).

²Pearson correlation coefficient *r* value.

Table 3. Pen body weight gain, lesion scores, and peak oocyst shedding following *Eimeria* challenge, experiment 3.¹

	Pen BWG (kg)		LS		OPG, ×10 ³	
	Day 14–19	Day 19–29	Day 19	Day 29	Day 20 (6 DPI)	Day 21 (7 DPI)
NC	4.25 ± 0.24	9.07 ± 0.67	0.45 ± 0.11 ^b	0.25 ± 0.10	ND ^{2,b}	ND
CC	4.09 ± 0.27	8.11 ± 0.69	0.90 ± 0.14 ^a	0.60 ± 0.15	148.10 ± 37.75 ^a	375.46 ± 91.42 ^a
<i>P</i> -value	NS	NS	0.033	NS	0.030	0.0261
	Day 14–19 BWG vs. day 19 LS		Day 14–19 BWG vs. day 21 OPG		Day 19 LS vs. day 20 OPG	
<i>r</i> ³	0.42		-0.93		-0.79	

^{a,b}Mean values with different superscript letters within a column indicate a significant difference ($P < 0.05$).

¹The nonchallenged control (NC) birds did not receive any treatment or challenge. Challenged control (CC) birds received 20,000 oocysts/bird of *Eimeria maxima* via oral gavage on day 14. Body weight was measured in grams per individual bird on day 14, day 19, and day 29 and then totaled per pen. On day 19 and day 29, 5 birds per pen, for a total of 20 birds per treatment, were evaluated for macroscopic lesions. Lesion scores (LS) were evaluated and scored on day 19 and day 29. Fecal samples were collected from each pen and then enumerated to determine oocysts per gram of feces (OPG) on day 21, 7 D post challenge (DPI). Data presented as mean ± SE (n = 4 pens/treatment).

²ND = none detected.

³Pearson correlation coefficient *r* value.

differences were detected at day 21, with $14.12 \times 10^3 \pm 4.89 \times 10^3$ OPG, and at day 25 with $27.44 \times 10^3 \pm 6.74 \times 10^3$ OPG in collected fecal samples. A very high positive correlation was observed between day 21 OPG and average day 21 LS ($r = 0.86$), which confirmed increased oocyst shedding on the same day intestinal damage occurred because of enterocyte rupture and oocyst release. However, a high negative correlation was observed between day 21 OPG and day 16–21 BWG ($r = -0.69$). This reflected expected decreased BWG in relation to increased OPG. However, moderate negative correlation was detected between day 16–21 BWG and average day 21 LS ($r = -0.49$), which reflected a weaker correlation between BWG and LS than between OPG and BWG or LS. The extremely low LS would likely not be considered biologically relevant despite

statistical differences, but the effect of *Eimeria* on BWG and fecal oocysts was more evident.

Lesion scores can provide information on *Eimeria* species responsible for infection, as well as the degree of intestinal damage at a particular moment, but the limited window in which to observe peak incidence of lesions is relatively narrow and dependent upon the incubation period of the species (Chapman et al., 2013). In turkeys, the window of macroscopically visible lesions was shown to be around a 3-D span, typically 5 to 7 DPI, with some variation in lesions observed across those days (Vrba and Pakandl, 2014). Lesion scores have also been shown to be affected by isolate, resulting in score variability and infection location based on *Eimeria* strains present (Barrios et al., 2017; El-Sherry et al., 2019), limiting the information they provide about infection severity and efficacy of treatment. Therefore, the incorporation

Table 4. Body weight gain, lesion scores, and shedding peaks after *Eimeria* challenge in turkeys on day 16, experiment 4.¹

BWG (g)	Day 16–21		Day 21–28		
NC	204.67 ± 3.56 ^a		395.57 ± 4.37 ^a		
CC	192.52 ± 4.07 ^b		307.26 ± 6.47 ^b		
<i>P</i> -value	<0.0001		<0.0001		
LS	Day 21 Duodenum	Day 21 Ceca	Day 28 Duodenum	Day 28 Ceca	
NC	0.00 ± 0.00 ^b	0.20 ± 0.09 ^b	0.03 ± 0.03 ^b	0.23 ± 0.08	
CC	0.13 ± 0.06 ^a	0.63 ± 0.17 ^a	0.23 ± 0.08 ^a	0.33 ± 0.09	
<i>P</i> -value	0.039	0.027	0.023	NS	
OPG, ×10 ³	Day 21 (5 DPI)	Day 22 (6 DPI)	Day 25 (9 DPI)	Day 26 (10 DPI)	
NC	ND ^{2,b}	ND	ND ^b	ND	
CC	14.12 ± 4.89 ^a	41.92 ± 17.24	27.44 ± 6.74 ^a	32.62 ± 16.14	
<i>P</i> -value	0.0344	NS	0.0096	NS	
	Day 16–21 BWG vs. Day 21 LS		Day 16–21 BWG vs. day 21 OPG		Day 21 LS vs. day 21 OPG
<i>r</i> ³	-0.49		-0.69		0.86

^{a,b}Mean values with different superscript letters within a column indicate a significant difference ($P < 0.05$).

¹Treatment groups varied based on the administration of a coccidial challenge comprised of *Eimeria adenoides* (EAd) and *Eimeria meleagridis* (EMel). On day 16, turkeys in the challenged control group (CC) were administered 6,250 EAd and 25,000 EMel oocysts each, whereas the nonchallenged (NC) birds did not receive any treatment. Body weight gain was measured in grams from day 16 through day 28. Lesion scores (LS) were evaluated and scored on day 21 and day 28 in the duodenum and ceca of the turkeys. Fecal collection was completed daily from floor pens, and then enumerated to determine oocysts per gram of feces (OPG). Peak shedding occurred on day 21, day 22, day 25, and day 26 at 5 D post infection (DPI), 6 DPI, 9 DPI, and 10 DPI, respectively. Data presented as mean ± SE (n = 6 pens/treatment).

²ND = none detected.

³Pearson correlation coefficient *r* value.

of LS should be used in conjunction with other measures of coccidial infection, such as OPG, to provide an accurate representation of the effects of certain prevention or treatment methods.

Fecal shedding of oocysts represents another parameter for measuring the disease impact of *Eimeria*. An increase in fecal shedding of oocysts has been demonstrated to be in direct proportion to pathogen ability to replicate within the GIT, but not in direct proportion to infection level, also considered the “crowding effect” (Zhu et al., 2000; Williams, 2001). However, OPG could still be deemed valuable because of its ability to project the level of replication within the bird, pen, or flock, and could provide additional insight into the severity of the infection when no difference in BWG or LS was observed. The utilization of OPG as a tool to detect parasite load of the flock is not new and has been used to categorize flocks at risk of decreased performance levels (Haug et al., 2008). This decreased performance was not evident during experiment 2, but the performance changes may have been masked by the OPG presence in both the NC and CC, which demonstrated the importance of not only relying on performance parameters, but including OPG as well.

Although all of the parameters discussed, BWG, LS, and OPG, are not often measured within the same experiment, some combination has often been used to determine effectiveness of certain treatment strategies (Lillehoj and Choi, 1998; Dalloul et al., 2003; Lee et al., 2007, 2009, 2010; Haug et al., 2008; Yim et al., 2011). The incorporation of OPG into any test, whether to test prophylactic or therapeutic strategies, can provide a level of insight into the effectiveness of reducing parasite load, or eliminating the parasite altogether. In a study completed by Dalloul and coauthors in 2003, a *Lactobacillus*-based probiotic evaluated oocyst shedding to confirm improved resistance to EAd. Not only did they find a reduction in oocyst shedding in the probiotic group but were also able to conclude that the probiotic provided an immunoregulatory effect, measured by intestinal intraepithelial lymphocytes and its surface markers (Dalloul et al., 2003). By incorporating oocyst shedding, they were able to assess the relative impact of the probiotic on pathogen load, even though the protozoa were not completely eliminated by incorporation of the probiotic. In an experiment completed more recently by Yim et al. (2011), BWG, LS, and oocyst production per bird were among the parameters measured to test a prophylactic treatment method on EMax infection. Among EMax-infected groups, which included 3 treatment groups and a CC, BWG did not differ between the groups, and LS only differed between the CC and the highest inclusion level of the product (Yim et al., 2011). However, differences in oocyst shedding were clearly observed when all 3 inclusion levels of the product were compared with CC (Yim et al., 2011). Although the BWG and LS can sometimes be understated, fecal shedding provided information on how the parasite load was affected by treatment. Several drugs have been established to have an effect on multiple stages of the *Eimeria* life cycle, affecting OPG

(Chappel, 1979; Smith et al., 1981; Long and Jeffers, 1982; Mehlhorn et al., 1983), with some ionophores additionally depressing BWG (McDougald and McQuiston, 1980). However, in the presence of *Eimeria* challenge, a reduction in OPG was observed in cases of both limited and improved growth (Weppelman et al., 1977; Braunius, 1985). The discovery of new treatment methods that have an impact on the replication stages can take advantage of OPG as a measurement for their effectiveness.

The importance of comprehensive measurements on treatment efficacy was clearly presented in these experiments, where the incorporation of OPG as a test parameter provided information on the disease impact within birds that was sometimes underestimated by BWG and LS data. Fecal shedding of oocysts can be used to provide a measure of infection severity and determine the success of intervention strategies on peak shedding and recovery. The measurement of OPG can be used to detect an impact on the replication cycle of oocysts that may not otherwise affect LS or BWG data when determining the effectiveness of treatment strategies. Finally, the inclusion of OPG as a standard test parameter for *Eimeria* infection studies and in the field provides a global description of disease that may not be observed with BWG and LS alone, particularly in cases of subclinical infection levels.

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