Effects of administration of an *in ovo* coccidiosis vaccine at different embryonic ages on vaccine cycling and performance of broiler chickens[1,2,3]

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ABSTRACT Use of a live coccidiosis vaccine has become an increasingly common method to control coccidiosis, especially in antibiotic-free broiler production. The Inovocox EM1 vaccine (**EM1**) is recommended for the vaccination of embryonated broiler hatching eggs between 18.0 and 19.0 d of incubation (**doi**). This allows for earlier acquisition of immunity to wild-type coccidia. However, it is unclear whether the difference in embryo age at the time of *in ovo* injection can influence the effect of the vaccine during grow-out as well as if the growth performance of broiler chickens is affected. Therefore, the objective of the study was to evaluate the effects of 2 injection ages (18.5 and 19.0 doi) and 3 injection types (noninjected, diluent, and vaccine) in a 3 × 2 factorial design, consisting of 10 replicates per treatment (60 treatment-replicate groups). There was a significant effect of injection age on BW at 0, 14, and 35 d after hatch, with a difference in the BW of birds belonging to the 18.5 and 19.0 doi groups up to day 35 after hatch. There was a significant effect of injection type on BW gain, feed intake, and FCR between 0 and 28 d after hatch. Between 0 and 35 d, FCR was lower in the vaccine-injected group in comparison with the noninjected and diluent control groups. Furthermore, total intestine coccidia and lesion indices were higher in the vaccine-18.5 treatment group in comparison with the diluent-18.5 treatment group at 28 d. In conclusion, hatchling weight was affected by injection age, and this subsequently affected growth performance. Furthermore, intestinal coccidia cycling peaked at 28 d, resulting in a reduction in growth performance through 28 d and subsequent compensatory growth by 35 d. There was no significant difference in coccidiosis cycling between the vaccine-18.5 and vaccine-19.0 doi treatment combination groups.

Key words: broiler chickens, coccidiosis vaccine, histology, performance

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INTRODUCTION

Coccidiosis is a host-specific parasitic disease caused by *Eimeria* spp. In broiler production, the disease causes high economic losses which are associated with increased medication cost and decreased flock performance (Price, 2012). The negative effect on performance stems from excessive coccidia cycling and intestinal lesions resulting in impaired nutrient absorption, low caloric conversion, and poor growth (Williams, 2005; McDougald et al., 2008). Furthermore, coccidiosis is reported to be a major predisposing factor to necrotic enteritis that is caused by the proliferation of pathogenic strain of *Clostridium perfringens* (Opengart et al., 2008; Moore, 2016). A survey of broiler production veterinarians in the United States indicates that coccidiosis and necrotic enteritis are the 2 most important diseases that affect broilers (Burleson, 2018). The degree of pathogenicity of coccidiosis can be measured by parameters such as performance, intestinal lesions, morbidity, and mortality (Johnson and Reid, 1970; Opengart et al., 2008).

Traditionally, coccidiosis prevention is achieved by using in-feed anticoccidials (i.e., polyether ionophores, or chemicals). Live coccidiosis vaccines are also common prevention strategies in broiler production programs (Williams, 2002), especially those which do not use antibiotics (Jenkins et al., 2017). In addition, more than

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one-third of broiler producers in the United States utilize a coccidiosis vaccine either as part of a rotation program or bioshuttle programs (Parent et al., 2018). Live coccidiosis vaccines are commonly applied through spray cabinets or by the *in ovo* injection of embryos during incubation (Danforth, 1998; Chapman et al., 2002; Mathis et al., 2014). Currently, over 80% of U.S. broilers are *in ovo* vaccinated with Marek's disease vaccine (Wakenell et al., 2002). The Inovocox EM1 vaccine (**EM1**) is a nonattenuated coccidiosis vaccine that contains live oocysts of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*, and is recommended for injection of embryonated broiler hatching eggs between 18 and 19 d of incubation (**doi**). The EM1 vaccine, although "dormant" during embryonic development following its administration, begins to replicate around the time of hatch (Weber and Evans, 2003; Sokale et al., 2017). The application of small doses of vaccinal oocysts stimulates protective immunity, through repeated fecal-oral cycling (McDougald et al., 2008; Tewari and Maharana, 2011; Sokale et al., 2017).

Previous studies have shown that the ideal time for *in ovo* vaccination is during late-stage embryonic development when the amniotic fluid is at its maximum, which corresponds to an embryo physiological age between 17.5 doi and 19.0 doi +4 h (Williams, 2007; Sokale et al., 2020). In commercial hatcheries, *in ovo* vaccination is typically administered during embryo transfer from the incubator into the hatcher between 18.0 and 19.0 doi. It has been shown that the injection of broiler chicken embryos with EM1 at either 18.5 or 19.0 doi has no detrimental effect on hatchability or chick quality (Sokale et al., 2017, 2018). In addition, effects of the EM1 vaccine administered at 18.0 or 19 doi on live performance have been studied independently by different authors (Weber et al., 2004; Mathis et al., 2014). It is unclear as to whether or not the *in ovo* administration of EM1 at 18.5 or 19.0 doi produces differential outcomes related to vaccine oocyst cycling, which subsequently affect the growth of broilers.

Therefore, the objective of this study was to determine the effects of the EM1 injected at either 18.5 or 19.0 doi on intestinal pathogenicity and the posthatch performance of Ross × Ross 708 broiler chickens. To the authors' knowledge, this is the first report that provides information concerning the comparative effects of vaccine administration timing within the same incubation system on broiler performance.

MATERIALS AND METHODS

General

All experimental procedures were conducted under a protocol that was approved by the Institutional Animal Care and Use Committee of Mississippi State University. The experimental design was a 3 × 2 factorial consisting of 3 injection types and 2 injection ages. The injection types were noninjected control (**noninjected**), diluent-injected control (**diluent**), and vaccine-injected (**vaccine**), and the injection ages were 18.5 and 19.0 doi. This resulted in a total of 6 combination treatments with 10 replicates per treatment (60 treatment-replicates) in both the incubation (Sokale et al., 2020) and feeding phases of the study. To achieve the injection ages 18.5 and 19.0 doi, all hatching eggs were set 12 h apart in a Jamesway model PS 500 single stage incubator (Jamesway Incubator Co. Inc., Cambridge, Ontario, Canada) and simultaneously injected at 18.5 doi.

Broiler Rearing

On the day of hatch, 17 straight-run chicks were randomly selected, wing-banded, weighed, and placed in 60 floor pens, measuring 0.91 m × 1.22 m, within an environmentally controlled broiler house. Birds were placed in pens in a randomized complete block design, in which all 6 combination treatments were randomly represented in each of 10 replicate blocks. Birds were reared on fresh wood-shavings litter, and standard commercial lighting and temperature conditions until 35 d. Diets were formulated to meet or exceeded NRC (1994) recommendations through 35 d. Diets contained no in-feed anticoccidials or antibiotics. Birds were fed a starter (crumbled) diet from day 0 to 14, a grower (pelletized) diet from day 14 to 28 and a finisher (pelletized) diet from day 29 to 35. Bird number, BW, and feed weight on a pen basis were determined weekly from 0 to 35 d. Body weight gain, feed intake and feed conversion ratio (**FCR**) adjusted for mortality, were calculated and reported.

Histopathology Evaluation

At 4 different time points (14, 21, 28, and 35 d), one bird was randomly selected from each of 3 treatment groups within each of 5 replicate blocks (diluent-18.5, vaccine-18.5, and vaccine-19.0) for intestinal histopathology scoring. In this evaluation, the diluent control group rather than the noninjected control group was compared with the vaccine groups because of the similarities in the injection of the hatching eggs and a similar environment in the embryo (i.e., the diluent and vaccine groups had "substances" injected into them). Furthermore, for comparison, only a diluent group was used because no *Eimeria* was expected in the control groups. The selected birds were individually weighed and euthanized, and their intestinal tracts (duodenum, jejunum, and cecum) were collected and fixed in 10% buffered neutral formalin solution. The formalin-fixed intestine tissues were processed and examined in accordance with the method described by Sokale et al. (2019). Briefly, each intestinal segment was semiquantitatively scored for severity based on a lesion panel. Enteritis index (**EI**) panel consisted of crypt hyperplasia, cystic crypts, villus damage, inflammation, dysbacteriosis, increased mucus, necrosis, and increased inflammatory cells. The severity of the EI was scored 0, normal; 1, minimal severity; 2, mild severity; 3, moderate; 4, marked;

and 5, severe. In addition, the duodenum, jejunum, and cecum were also scored for the degree of presence of *E. acervulina*, *E. maxima*, and *E. tenella* to determine a coccidia index (**CI**). The CI was scored on a scale of 1 to 4 as follows: 0, no coccidia observed;1, 0-20 coccidia; 2, up to 50 coccidia; 3, up to 75 coccidia; 4, up to 100 coccidia; 5, >100 coccidia. A total lesion index was calculated by summing the enteritis and coccidia indices for each section of intestine. Each individual intestinal segment (duodenum, jejunum, and cecum) of the 5 birds per treatment group per time point was scored and the mean of each index for each segment was calculated and reported. In addition, the scores for each individual intestine segment were summed to derive a total intestine score for each index. The mean of the total intestine scores by treatment combination group and time point for each index was reported. The mean per treatment group for all time points combined for each index was also reported. All scoring was accomplished with no knowledge of treatment group by the pathologist.

Statistical Description

A randomized complete block design was used, with data arranged in a 3 × 2 factorial design to evaluate the main and interaction effects of injection type and injection age on all performance variables. Data analysis was performed by two-way ANOVA with the main and interaction effects viewed as fixed effects and block as a random effect. Histology scores (enteritis, coccidia, and total lesion indices) were statistically analyzed using the nonparametric Kruskal-Wallis test, with Dunn's test for nonparametric pairwise multiple comparisons performed as a post hoc test for treatment comparison. Least-square means were compared in the event of significant global effects (Steel and Torrie, 1980). All variables were analyzed using the MIXED procedure of SAS software 9.3 (SAS Institute, 2012). Global and least-squares means differences were considered significant at $P \leq 0.05$.

RESULTS

The means for the main and interactive effects of injection type and injection age on performance are presented in Tables 1 and 2. There was a significant injection type and injection age interaction for BW at d 0 after hatch (Table 1). The BW of birds was highest in the noninjected-18.5 and diluent-18.5 treatment groups, and lowest in the noninjected-19.0 and diluent-19.0 treatment groups, with the vaccine-18.5 treatment group being intermediate. There were no interactive effects of injection age and injection type on the performance variables examined throughout the grow-out period, except for feed intake at day 35. However, there was a significant main effect of injection age on BW at 0, 14, and 35 d (Table 1). At day 0 after hatch, BW was higher in birds belonging to the 18.5 doi group in comparison with those in the 19.0 doi group. However, at 14 and 35 d, BW was higher in the 19.0 doi group in comparison with those in the 18.5 doi group. At 28 d, there was a significant main effect of injection type on BW, with those in the vaccine group displaying the lowest BW in comparison with the noninjected and diluent control groups (Table 1). Similarly, at 0 to 28 d, there was a significant main effect of injection type on BW gain, feed intake, and FCR (Table 2). The BW gain and feed intake of the birds were lower in the vaccine group in comparison with the noninjected and diluent control groups. However, FCR was improved in the vaccine group in comparison with the noninjected and diluent control groups. There was a significant main effect of injection age on BW gain and feed intake between day 0 and 14, and feed intake between 0 and 28 d (Table 2). In all these intervals, the performance variables were higher in birds belonging to the 19.0 doi group than in those in the 18.5 doi group. At 0 to 35 d, there was a significant main effect of injection age on BW gain (Table 2). The BW gain of birds in the 19.0 doi group was higher than those in the 18.5 doi group. In addition, there was a significant injection type and injection age interaction for feed intake (Table 2). Feed intake was highest in the diluent-19.0 and noninjected-19.0 treatment groups, and lowest in the vaccine-18.5 and diluent-18.5 treatment groups. Furthermore, there was a significant main effect of injection type on FCR, with the vaccine group showing a lower FCR in comparison with the noninjected and diluent control groups (Table 2).

There was a significant treatment effect for EI in the duodenum at 21 and 35 d. Duodenal EI was higher in the vaccine-19.0 treatment group than in the diluent-18.5 treatment group, with those in the vaccine-18.5 treatment group being intermediate. There was a significant treatment effect on the duodenal coccidia and total lesion indices at 28 d. The duodenal coccidia and total lesion indices were higher in birds in the vaccine-19.0 treatment group than in those in the diluent-18.5 control group, with those in the vaccine-18.5 treatment group being intermediate. Similarly, there was a significant treatment effect on the cecal coccidia and total lesion indices at 28 d. Both the coccidia and total lesion indices were higher in the vaccine-18.5 treatment group than in the diluent-18.5 control group, with the vaccine-19.0 treatment group being intermediate. In the jejunum, only the EI was significantly different among the treatment groups at 28 d. The jejunal EI was significantly higher in the vaccine-18.5 treatment group than in the diluent-18.5 control group, with the vaccine-19.0 treatment group being intermediate. The means of all the indices for each intestinal segment are presented in Table 3.

For evaluations based on the sum of scores for total intestine indices within each time point, a significant treatment for CI was observed only at 28 d, with the vaccine-18.5 treatment group showing the highest CI in comparison with the diluent-18.5 control group, and with the vaccine-19.0 treatment group being intermediate (Figure 1). There was no significant treatment effect on the EI (Figure 2) and total lesion index (Figure 3) at

Table 1. Performance variables of broiler chickens from 0 to 35 d of age after the administration of an *in ovo* coccidiosis vaccine at different embryonic ages.

Treatments	Injection type	Injection age	Body weight (g)			
			Day 0	Day 14	Day 28	Day 35
	Noninjected control	18.5	48[a]	453	2,078	2,242
		19.0	46[c]	464	2,033	2,303
	Diluent-injected control	18.5	48[a]	436	1,822	2,213
		19.0	46[c]	479	1,959	2,295
	Vaccine-injected	18.5	47[b]	438	1,161	2,179
		19.0	46[b,c]	463	1,223	2,261
	SEM		0.213	7.38	186	57.5
Injection type	Noninjected control		47	458	2,056[a]	2,273
	Diluent-injected control		47	458	1,890[a]	2,254
	Vaccine-injected		47	450	1,192[b]	2,220
	SEM		0.151	5.22	132	40.7
Injection age	18.5		48[a]	442[b]	1,687	2,211[b]
	19		46[b]	468[a]	1,738	2,287[a]
	SEM		0.123	4.26	108	33.2
P-values	Injection type		0.299	0.475	<0.001	0.897
	Injection age		<0.001	<0.001	0.738	0.011
	Injection type × Injection age		0.010	0.100	0.887	0.632

[a-b]Means within a column with no common superscript differ significantly ($P \leq 0.05$). For the calculation of means; injection type (n = 20), injection age (n = 30), injection type × injection age (n = 10) with pen block as a replicate unit.

14, 21, 28, or 35 d. Furthermore, for evaluations based on the sum of scores for total intestine indices for all the time points within each treatment group, there was a significant treatment effect on the coccidia (Figure 4) and total lesion indices (Figure 5). Coccidia and total lesion indices were higher in birds belonging to the vaccine-19.0 treatment group than in birds in the diluent-18.5 control group, with birds in the vaccine-18.5 treatment group being intermediate. There was no significant treatment effect for EI (Figure 6).

DISCUSSION

A companion study which evaluated the effects of 2 injection ages and injection types on hatchability and chick quality has been previously published by Sokale et al. (2020).

Table 2. Performance variables of broiler chickens from 0 to 35 d of age after the administration of an *in ovo* coccidiosis vaccine at different embryonic ages.

Treatments	Injection type	Day 0–14				Day 0–28			Day 0–35		
		IAN	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR
	Noninjected control	18.5	405	431	1.06	1,310	1,815	1.39	2,195	3,394[b]	1.55
		19.0	418	437	1.05	1,402	1,891	1.35	2,259	3,584[a]	1.59
	Diluent-injected control	18.5	388	410	1.06	1,265	1,763	1.39	2,164	3,213[c]	1.49
		19.0	433	453	1.05	1,408	1,875	1.33	2,249	3,652[a]	1.62
	Vaccine-injected	18.5	391	403	1.03	1,275	1,750	1.37	2,131	3,207[c]	1.51
		19.0	416	439	1.05	1,307	1,754	1.34	2,214	3,311[b,c]	1.50
	SEM		7.39	10.10	0.011	0.190	28.7	0.068	57.54	47.2	0.029
Injection type	Noninjected control		411	434	1.06	1,356[a]	1,753[a]	1.29[a]	2,227	3,489[a]	1.57[a]
	Diluent-injected control		411	431	1.05	1,336[a]	1,718[a]	1.29[a]	2,207	3,433[a]	1.56[a]
	Vaccine-injected		403	421	1.04	1,292[b]	1,645[b]	1.27[b]	2,173	3,259[b]	1.50[b]
	SEM		5.22	7.14	0.008	0.134	20.5	0.058	40.68	33.4	0.020
Injection age	18.5		395[b]	415[b]	1.05	1,384	1,776[b]	1.28	2,163[b]	3,272[b]	1.51
	19.0		422[a]	443[a]	1.05	1,373	1,836[a]	1.34	2,241[a]	3,516[a]	1.57
	SEM		4.27	5.83	0.006	0.110	17.0	0.083	33.22	27.2	0.017
P-values	Injection type		0.497	0.382	0.494	<0.001	0.003	0.002	0.8961	<0.001	0.013
	Injection age		<0.001	0.001	0.893	0.897	0.004	0.710	0.010	<0.001	0.415
	Injection type × injection age		0.097	0.164	0.163	0.803	0.192	0.863	0.627	0.002	0.375

[a-b]Means within a column with no common superscript differ significantly ($P \leq 0.05$). For the calculation of means; injection type (n = 20), injection age (n = 30), injection type × injection age (n = 10) with pen block as a replicate unit.

Table 3. Mean indices at day 14, 21, 28, and 35 in broilers injected with the coccidiosis vaccine at 18.5 and 19.0 d of incubation.

			Treatment groups				
Intestine segment	Age (day)	Variables	Diluent 18.5	Vaccine 18.5	Vaccine 19.0	SEM	*P*-value
Duodenum	14	Cocci index	1.00	1.00	1.00	1.00	1.00
		Enteritis index	1.00	1.11	1.17	0.051	0.091
		Total lesion index	1.00	1.06	1.09	0.025	0.091
	21	Cocci index	1.00	1.00	1.00	1.00	1.00
		Enteritis index	1.06[b]	1.14[a,b]	1.30[a]	0.044	0.009
		Total lesion index	1.03	1.10	1.11	0.028	0.138
	28	Cocci index	1.00[b]	1.40[a,b]	1.80[a]	0.183	0.029
		Enteritis index	1.20	1.29	1.23	0.069	0.686
		Total lesion index	1.10[b]	1.34[a,b]	1.51[a]	0.107	0.053
	35	Cocci index	1.00	1.00	1.40	0.231	0.396
		Enteritis index	1.13[b]	1.29[a,b]	1.32[a]	0.047	0.028
		Total lesion index	1.06	1.14	1.36	0.129	0.281
Jejunum	14	Cocci index	1.00	1.00	1.00	1.00	1.00
		Enteritis index	1.20	1.13	1.17	0.041	0.531
		Total lesion index	1.10	1.07	1.08	0.02	0.531
	21	Cocci index	1.00	1.00	1.25	0.117	0.309
		Enteritis index	1.23	1.33	1.38	0.063	0.332
		Total lesion index	1.12	1.17	1.31	0.064	0.155
	28	Cocci index	1.00	1.25	1.40	0.189	0.352
		Enteritis index	1.27[b]	1.58[a]	1.4[a,b]	0.069	0.033
		Total lesion index	1.13	1.42	1.40	0.105	0.16
	35	Cocci index	1.00	1.00	1.40	0.346	0.397
		Enteritis index	1.29	1.25	1.29	0.073	0.905
		Total lesion index	1.13	1.13	1.45	0.189	0.422
Cecum	14	Cocci index	1.00	1.00	1.20	0.115	0.397
		Enteritis index	1.12	1.20	1.48	0.104	0.074
		Total lesion index	1.06	1.10	1.34	0.102	0.153
	21	Cocci index	1.40	1.60	1.75	0.488	0.891
		Enteritis index	1.40	1.32	1.30	0.067	0.575
		Total lesion index	1.40	1.46	1.53	0.264	0.951
	28	Cocci index	1.00[b]	2.75[a]	1.60[a,b]	0.38	0.032
		Enteritis index	1.48	1.50	1.56	0.097	0.837
		Total lesion index	1.24[b]	2.13[a]	1.58[a,b]	0.192	0.033
	35	Cocci index	1.00	1.20	1.20	0.163	0.619
		Enteritis index	1.36	1.36	1.52	0.067	0.195
		Total lesion index	1.18	1.28	1.36	0.094	0.423

[a-b]Means within a row with no common superscript differ ($P \leq 0.05$). Results are reported as means of 5 birds per treatment for each of 4 time points (15 birds/time point) with bird as the replicate unit.

Although injection types (vaccine and diluent injection) did not affect broiler hatchability, chick quality characteristics were affected by injection age (injection at 18.5. and 19.0 doi). The effects of both injection ages and injection types on broiler performance were evaluated in the present study. Effects of the live nonattenuated EM1 vaccine

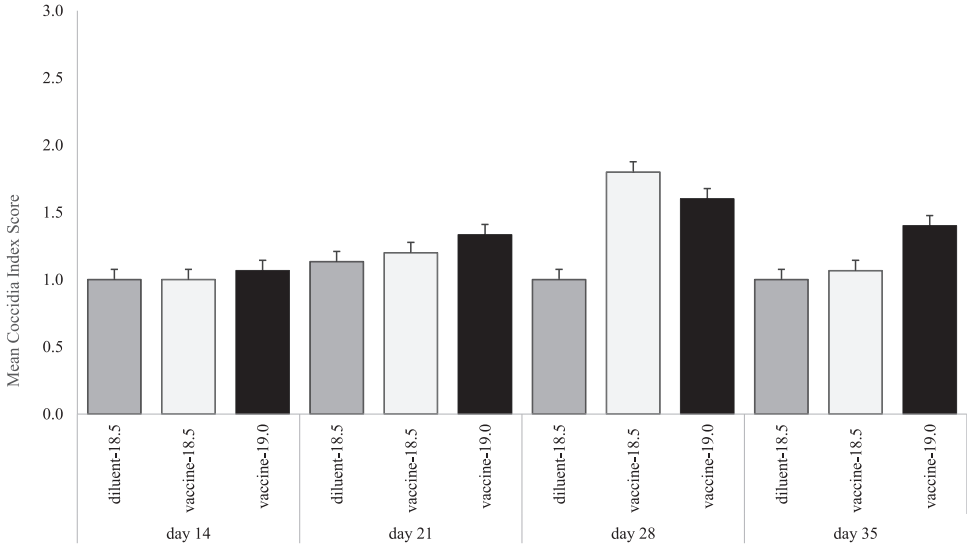

Figure 1. Total intestine coccidia index by treatment for each time point (day 14, 21, 28, and 35). Data from 5 birds per treatment (diluent-18.5, vaccine-18.5, vaccine-19.0) was used to calculate means. Coccidia score of 1 to 4 for *Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella.* [a-b]Means with no common superscript differ ($P \leq 0.05$).

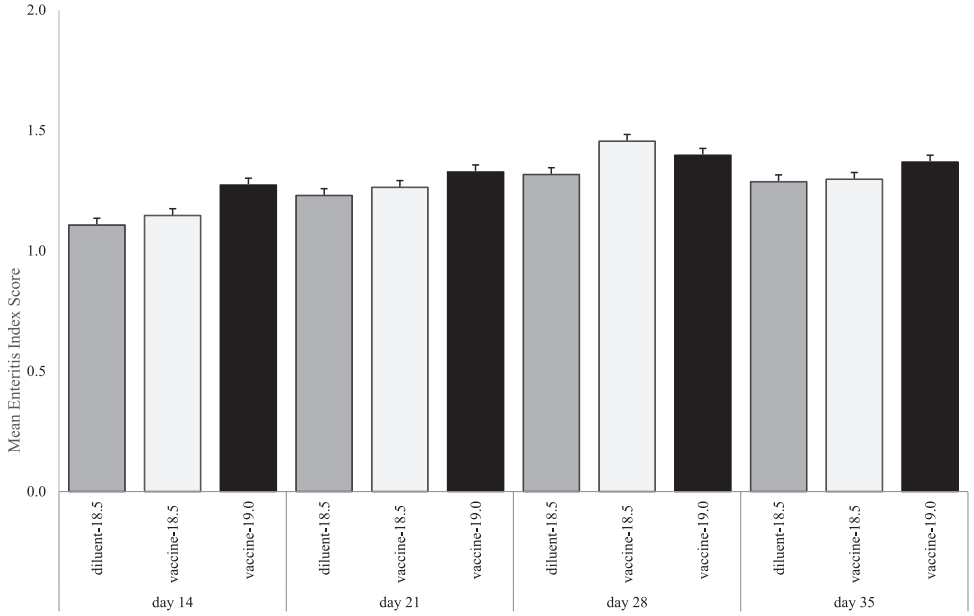

Figure 2. Total intestine enteritis index (score for inflammation and repair) by treatment for each time point (day 14, 21, 28, and 35). Data from 5 birds per treatment (diluent-18.5, vaccine-18.5, vaccine-19.0) was used to calculate means. Enteritis lesion panel score from 1 to 4. No significant difference was observed among treatments within each time point.

administered at 18 or 19 doi on live performance have been studied independently by different authors (Weber et al., 2004; Mathis et al., 2014). However, to the authors' knowledge, this is the first study which examines the effect of the EM1 vaccine administered at 2 time points together in a single study, on coccidia cycling and growth performance.

The shift of the U.S. poultry industry toward antibiotic-free broiler production has resulted in changes or modifications in the use of chemical and ionophore anticoccidials for the control of coccidiosis. This is because they either develop varying levels of resistance due to prolong use, as in the case of chemical anticoccidials (Chapman, 1997) or they are deemed unacceptable based on their classification as an antibiotic as in the case of ionophores (Peek and Landman, 2011). Therefore, to effectively control coccidiosis, most U.S. poultry producers now use vaccines as part of a rotation program or in a bioshuttle program. Coccidiosis vaccines which contain live *Eimeria* oocysts are applied early in the life of the bird to facilitate the development

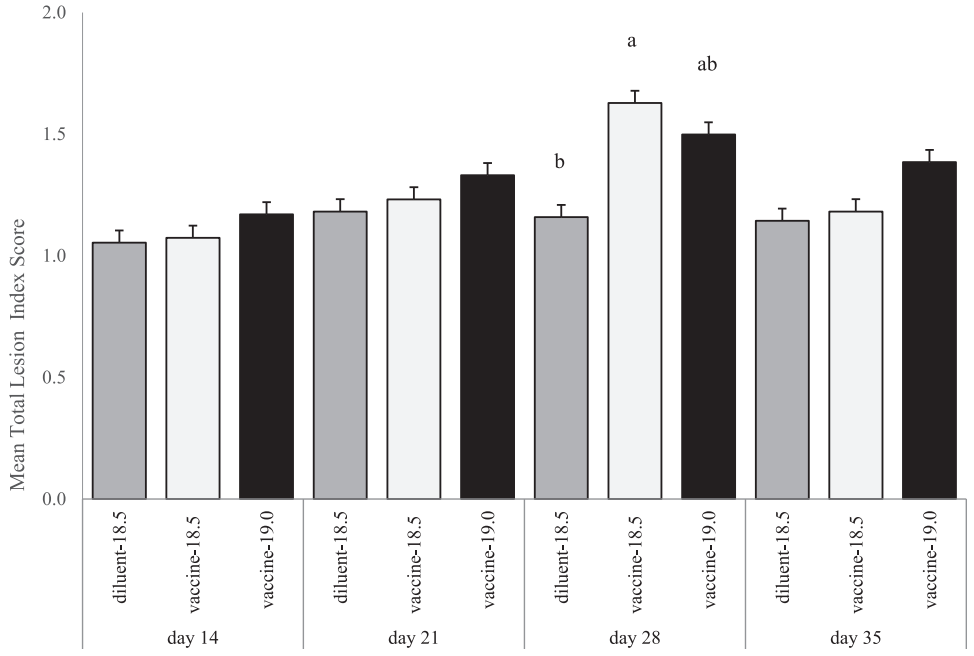

Figure 3. Total intestine lesion index (inflammation, repair, and coccidia) by treatment for each time point (day 14, 21, 28, and 35). Data from 5 birds per treatment (diluent-18.5, vaccine-18.5, vaccine-19.0) was used to calculate means. Total lesion index scores from 1 to 4. [a-b]Means with no common superscript differ ($P \leq 0.05$).

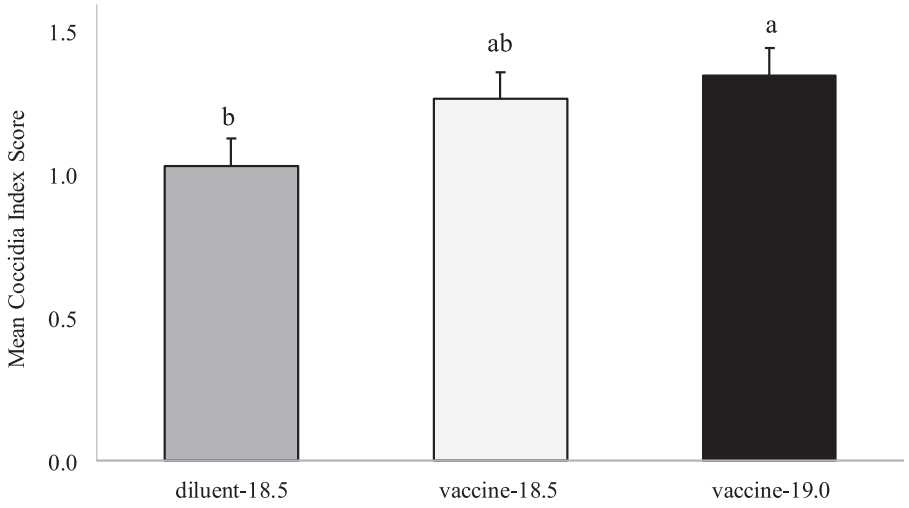

Figure 4. Total intestine coccidia index by treatment for all time points combined. Data from 20 birds per treatment (diluent-18.5, vaccine-18.5, vaccine-19.0) was used to calculate means. Coccidia score of 1 to 4 for *Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella.* [a-b]Means with no common superscript differ ($P \leq 0.05$).

of their immunity against wild-type *Eimeria* spp. after adequate oocyst cycling (Chapman, 2000; Chapman et al., 2013; Price et al., 2016). Previous independent studies have examined effects of the *in ovo* injection of infective stages of *Eimeria* at 18.0 doi (Weber and Evans, 2003, 2004) or the EM1 vaccine at 18.5 doi (Sokale et al., 2017). In those reports, there were no observed effects on chick BW. In the present study, chick BW at day 0 after hatch was not affected by injection type. Although, there were interactive effects of injection type and injection age on BW at day 0 after hatch, the BW of chicks in the vaccine-18.5 and vaccine-19.0 treatment groups were not significantly different from the noninjected and diluent control groups. This may indicate that differences in chick BW were largely due to differences in injection age (18.5 and 19.0 doi).

In the incubation phase of the study, hatching eggs were set 12 h apart but were all injected at 18.5 doi (Sokale et al., 2020). Incubation length for both groups extended to approximately 21.0 doi. A higher hatchling BW was observed in the 18.5 doi group than in the 19.0 doi group. The difference in the BW of the chicks may be primarily attributed to the fact that the chicks were at different stages of physiological development at the time of injection and hatch, potentially due to several contributory factors which have been described in the literature. During incubation, temperature, humidity, air flow, and differences in embryonic heat production all contribute to the development of the embryo, and subsequently affect posthatch chick quality and performance (Molenaar et al., 2011; Pulikanti et al., 2013; Sokale et al., 2020). Furthermore, chicks in the 18.5 doi group exhibited a partial delay in yolk uptake/utilization,

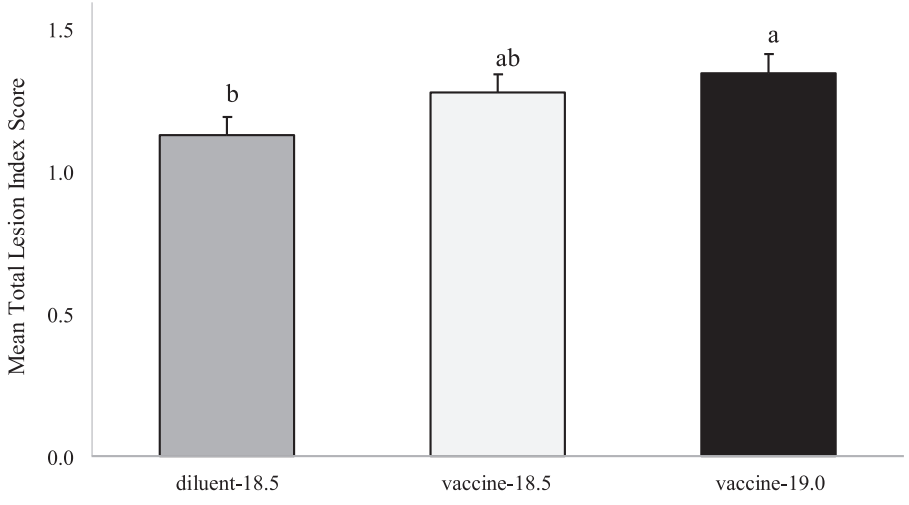

Figure 5. Total intestine lesion index (inflammation, repair, and coccidia) by treatment for all time points combined. Data from 20 birds per treatment (diluent-18.5, vaccine-18.5, vaccine-19.0) was used to calculate means. Total lesion index scores from 1 to 4. [a-b]Means with no common superscript differ ($P \leq 0.05$).

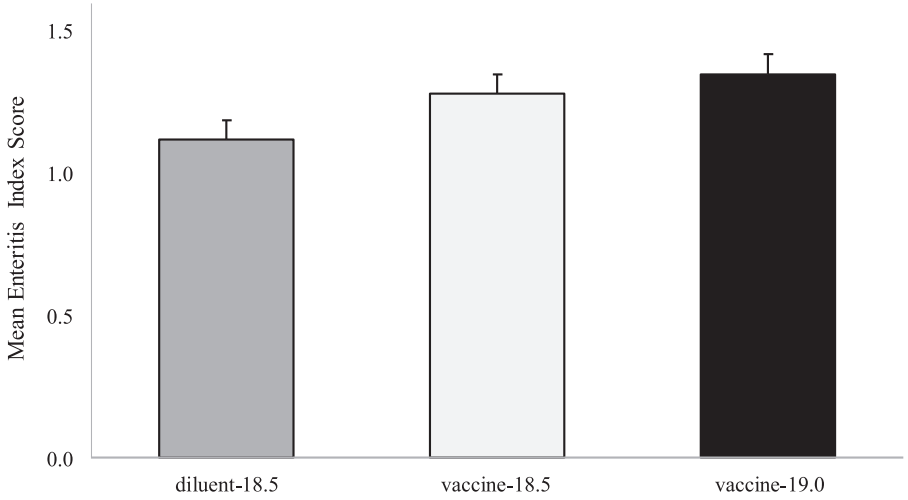

Figure 6. Total intestine enteritis index (score for inflammation and repair) by treatment for all time points combined. Data from 20 birds per treatment (diluent-18.5, vaccine-18.5, vaccine-19.0) was used to calculate means. Enteritis lesion panel score from 1 to 4. No significant difference was observed among treatment groups.

resulting in a delay in feed intake. By contrast, chicks in 19.0 doi group exhibited complete yolk uptake/utilization and were able to initiate feed intake sooner, resulting in accelerated growth. Zhai et al. (2011b) showed that the injection of carbohydrates reduced yolk absorption at 19.5 and 21.0 doi and consequently reduced the yolk-free BW of hatchlings. Furthermore, it's been shown that delayed access to feed or delayed feed intake after hatch can result in adverse effects on posthatch growth performance (Bigot et al., 2003; Gonzales et al., 2003). In the present study, BW differences due to injection age which were observed at day 0 after hatch, extended up to day 35, with birds in the 19.0 doi group having a greater BW at day 14 and 35 in comparison with those in the 18.5 doi group. The increase in BW in that group was also accompanied by an increase in feed intake and BW gain between day 0 and 14 and between day 0 and 35. This indicate that the 19.0 doi group may have experienced a complete utilization of their yolk stores and increased feed intake after placement, resulting in an increase in weight gain. Conversely, chicks belonging to the 18.5 doi group may have experienced a "restricted" feed intake due to suboptimal yolk utilization. Previous studies have shown that feed and water restriction can result in significant reductions in BW of broilers during grow-out (Stamps and Andrews, 1995; Vieira and Moran 1999; Peebles et al., 2005, 2017).

An effect of injection type on the performance variables was observed between day 0 and 28 and between day 0 and 35. At day 28, a reduction in BW, BW gain, and feed intake were observed in birds belonging to the vaccine treatment group, and this reduction coincided with higher coccidia and total lesion indices. The life cycles of *Eimeria* spp. include both the host and the environment (Chapman et al., 2002; Price, 2012). Although, vaccine application focuses on the control of parasites in the host, the development of immunity to *Eimeria* spp. depends on coccidia cycling, which is an interplay between the environment (i.e., oocyst sporulation) and the host (i.e., oocyst ingestion; Price et al., 2014). The development of immunity and the severity of coccidiosis is dependent on the number of sporulated oocysts ingested by the bird. The development of *Eimeria* spp. can be monitored by examining the intestinal tissue macroscopically for the presence of lesions that are indicative of coccidiosis (Johnson and Reid, 1970; Chapman 2002; Price, 2012). Previous studies in which the cycling pattern in coccidiosis-vaccinated birds were examined showed that peak cycling occurs around day 21 to 28 after hatch and in certain instances, cycling beyond day 28 after hatch has also been reported (Jenkins et al., 2017). Furthermore, Mathis et al. (2014) reported a higher level of oocyst shedding in litter at 21 d after hatch in EM1-vaccinated birds than in their control counterparts, with oocyst shedding continuing up to day 35 after hatch. In the present study, the highest CI was observed at 28 d, and was primarily associated with a higher index in the duodenum and cecum. The CI pattern observed in the present study suggests a cycling of the vaccine oocysts with a minimal level of influence by environmental oocysts. This is because the CI of birds in the control group was lower than those in the vaccine treatment groups throughout the study, which indicates that control birds did not ingest wild-type oocysts from the environment. This would have resulted in more intestinal lesions, because no protection against coccidiosis would have existed in birds belonging to the control group. This effect would be expected because new litter was utilized in this study. New litter does not provide the needed nutrients (moisture and relative humidity) needed for oocyst sporulation (Price et al., 2014). The resulting intestinal lesions from oocyst cycling (enteritis and total lesion indices) were also significantly increased at 28 d in the duodenum, jejunum, and cecum. This further confirms that cycling of coccidia can damage the intestinal tissue, resulting in a reduction in growth performance.

By 35 d, the coccidia and total lesion indices of birds in the vaccine-18.5 and vaccine-19.0 treatment groups were not significantly different from those in the diluent-18.5 group, indicating that the birds may have undergone enough cycling to develop immunity to coccidiosis. Furthermore, the CI (all time points combined) was significantly higher in birds belonging to the vaccine-19.0 treatment group than the diluent-18.5 control group but was not different from birds in the vaccine-18.5 treatment group. Injection type affected FCR at 35 d, with birds in the vaccine group showing the lowest FCR. This indicated that vaccine application improved the growth performance of the birds despite a higher CI. Previous studies have shown that a depression in performance may occur during peak coccidiosis cycling, with a compensatory improvement in performance occurring later during grow-out (Williams and Gobbi, 2002; Mathis et al., 2014). These findings agree with this current study in which vaccine application resulted in a reduction in performance during cycling. However, BW and feed efficiency were improved by 35 d, which may indicate a compensatory effect in flock performance.

In conclusion, throughout the entire study, there were no differences in the coccidia, enteritis, and total lesion indices between the vaccine-18.5 and vaccine-19.0 treatment groups. However, quality was improved in chicks that had the advantage of an additional 12 h of incubation time (19.0 doi group), and this resulted in a difference in performance through 35 d. The growth performance of birds in the EM1-vaccinated group was reduced during peak coccidia oocyst cycling at 28 d, and although BWG at 35 d was not significantly different between birds in the diluent- and vaccine-injected treatment groups, FCR was improved in the vaccine group in comparison with both the control groups.

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DISCLOSURES

There are no conflicts of interest for this article (PSJ-D-20-010680).

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