The effect of dietary protein level on performance characteristics of coccidiosis vaccinated and nonvaccinated broilers following mixed-species *Eimeria* challenge

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ABSTRACT A series of experiments were conducted to investigate the effect of starter diet protein levels on the performance of broilers vaccinated with a commercially available live oocyst coccidiosis vaccine before subsequent challenge with a mixed-species *Eimeria* challenge. Data indicated that an increasing protein concentration in the starter diet improved broiler performance during coccidiosis vaccination. Prechallenge performance data indicated that vaccination could decrease BW and increase feed conversion ratio. The time period most important for the observed effects appeared to be between 13 and 17 d of age. This reduction in performance parameters of vaccinated broilers compared with nonvaccinated broilers was eliminated by the conclusion of the experiments (27 d) in the diet groups with higher protein. Vaccination was effective at generating protective immunity against *Eimeria* challenge, as evidenced by increased ($P < 0.05$) BW gain, improved feed conversion, reduced postchallenge mortality, and reduced lesion development in vaccinated broilers compared with nonvaccinated broilers. These observations support numerous other reports that confirm live oocyst vaccination can be used effectively as a preventive against avian coccidiosis in commercially reared broilers. More important, these findings suggest that reduced protein concentration of starter diets can lead to significant losses in broiler performance when using a vaccination program to prevent coccidiosis.

Key words: broiler, *Eimeria*, protein, vaccination

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INTRODUCTION

Coccidiosis is an intestinal disease of intensively reared livestock caused by protozoan parasites of the genus *Eimeria*. The disease causes intestinal epithelium lesions, reduced BW, reduced feed efficiency, and often overt morbidity and mortality (Guzman et al., 2003). The most important species of *Eimeria* that infect chickens worldwide and cause significant economic losses are *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria praecox*, *Eimeria necatrix*, and *Eimeria tenella* (Shirley et al., 2005). Infections with *E. acervulina*, *E. maxima*, and *E. tenella* are diagnosed frequently in intensively reared poultry (McDougald and Fitz-Coy, 2008). It has been estimated that coccidiosis costs the world's commercial chicken producers at least $800 million each year (Williams, 1998), with approximately 80% of this cost attributable to poor performance (Williams, 1999). As the world's poultry production continues to grow, so do concerns about the control of coccidiosis because it is extremely rare to find commercial chicken flocks unaffected by coccidia (Williams, 2002). Historically, the poultry industry has relied on anticoccidial drugs for the control of coccidiosis, but *Eimeria* has developed drug resistance against most anticoccidials used today (Chapman, 1997; Mathis and Broussard, 2006; Sharman et al., 2010). Consumers are becoming increasingly concerned about drug residues in poultry products (McEvoy, 2001), resulting in increased pressure from a percentage of consumers to remove all drugs from animal feeds. As such, there is a pressing interest in moving away from chemotherapeutic control of coccidiosis in favor of nonmedicated forms of control, such as vaccination (Williams, 2002).

Vaccination against coccidiosis is not a new concept and has been used in the poultry industry for more

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than 50 yr (Shirley et al., 2005). Live oocyst vaccination using attenuated and nonattenuated *Eimeria* is currently the only commercially available alternative to chemotherapy for control of coccidiosis in poultry. These vaccines provide immunity to coccidial infection when used under good rearing conditions (Shirley and Long, 1990). Despite the proven success of these vaccines in eliciting effective protection against coccidiosis in replacement and breeding flocks, they have not been universally accepted by the US poultry industry for meat-producing broiler and heavy roaster bird flocks (Danforth, 1998). The reluctance of broiler producers to adopt anticoccidial vaccination strategies is related to several reports on measured performance parameters associated with vaccination, BW gain, and feed efficiency. The performance of vaccinated broilers has not always equaled that of medicated broilers (Danforth, 1998; Williams, 2002). The reduced performance is related to mild coccidial infection associated with live oocyst vaccination. Increasing the protein level during periods of clinical coccidiosis has been shown to improve broiler performance (Sharma et al., 1973), but to date, such a strategy has not been evaluated during live oocyst vaccination. The objective of the current research was to evaluate the effect of dietary protein level on broiler performance and lesion development during live oocyst vaccination with Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ) and subsequent mixed-species *Eimeria* challenge.

MATERIALS AND METHODS

Within this study, a series of 3 experiments were designed to evaluate the effect of selected levels of dietary protein in starter diets on broiler performance and immunity generation while using a vaccination program for the prevention of coccidiosis. Growth parameters of vaccinated broilers were compared with nonmedicated, nonvaccinated broilers before and after a mixed-species *Eimeria* challenge. For all experiments, Cobb × Cobb straight-run broiler chicks obtained from a local integrator were provided age-appropriate supplemental heat and were given access to feed and water ad libitum. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Institutional Animal Care and Use Committee at Texas A&M University. Before chick placement, grow-out facilities were thoroughly cleaned and sanitized. Fresh pine shavings were used for bedding material. Pens were equipped with one 30-lb (13.6-kg) tube feeder and nipple drinkers. In experiments that included a mixed-species *Eimeria* challenge, dose titration of the challenge inoculum was performed before the experiment to identify a dose sufficient to cause identifiable gross lesions in vaccinated broilers. Oocysts used for clinical challenge were field-strain *Eimeria* isolated from commercial broiler rearing houses. Oocyst strains were maintained as individual species by propagation and passage according to the methods of Allen et al. (2000). Oocysts were stored in a 2.5% potassium dichromate solution and then enumerated before challenge.

Experiment 1

Experiment 1 was a randomized block design consisting of 5 dietary protein levels (20, 21, 22, 23, and 24%) with 8 replicates of each protein level, for a total of 40 pens. Each replicate contained 25 chicks, for a total of 1,000 chicks placed. Diets were formulated on an isocaloric basis, and careful consideration was given to maintaining constant amino acid-to-protein ratios throughout all 5 dietary treatments (Table 1). The 23% dietary treatment met or exceeded NRC (1994) specifications for a broiler starter diet. On the day of hatch, all chicks were individually weighed, wing banded, and vaccinated with a single vaccine dose of Coccivac-B (Intervet/Schering Plough Animal Health) by oral gavage. Once vaccinated, chicks were randomly assigned to treatment groups based on chick BW in a manner that ensured statistically similar BW at 1 d of age. Broiler chicks were reared for 21 d, at which time pen weights were taken and feed consumption was determined for calculation of the feed conversion ratio.

Experiment 2

Experiment 2 was a 3 × 2 × 2 factorial with the variables of 3 dietary protein levels (20, 22, and 24%), vaccination (vaccinated compared with nonmedicated, nonvaccinated), and a mixed-species *Eimeria* challenge (challenged and nonchallenged). This experimental design generated a total of 12 treatment groups, and each group was replicated in triplicate. Each replicate contained 25 chicks, for a total of 900 chicks placed. Dietary treatments were identical to those used in experiment 1 for the 3 protein levels selected (Table 1). On the day of hatch, chicks were individually weighed, wing banded, and assigned to treatment groups. Chicks assigned to the vaccinated treatment groups were vaccinated with Coccivac-B (Intervet/Schering Plough Animal Health) in a commercial spray cabinet according to the manufacturer's instructions. After spray vaccination, chicks were allowed to preen for 1 h before placement to allow for vaccine uptake. On d 21, pen weights were taken and feed consumption was determined for calculation of the feed conversion ratio. One-half of the treatment groups were challenged with a mixed-species challenge containing *E. acervulina* (6×10^5), *E. maxima* (4×10^5), and *E. tenella* (2×10^5) sporulated oocysts. On d 27 (6 d postchallenge), pen weights were taken, feed consumption was determined, and 10 broilers from each replicate were necropsied for quantitative assessment (scoring) of the development of intestinal lesions (Johnson and Reid, 1970).

Table 1. Calculated nutrient composition of experimental diets used in experiments 1 through 3

	Protein				
Item (% unless noted)	20.0%	21.0%	22.0%	23.0%	24.0%
Ingredient					
Corn	60.5	57.5	54.5	51.5	48.5
Soybean meal (48% CP)	31	33.6	36.2	38.8	41.4
Fat	4.6	5.0	5.4	5.8	6.2
DL-Methionine	0.14	0.15	0.17	0.18	0.20
Limestone	1.45	1.44	1.43	1.42	1.40
Monocalcium phosphate	1.59	1.57	1.56	1.54	1.52
Sodium chloride	0.46	0.46	0.46	0.46	0.46
Minerals[1]	0.05	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25	0.25
Nutrient					
Methionine	0.44	0.47	0.50	0.52	0.55
TSAA	0.78	0.82	0.86	0.90	0.94
Lysine	1.06	1.12	1.19	1.26	1.33
Threonine	0.75	0.79	0.83	0.87	0.91
Arginine	1.32	1.40	1.48	1.55	1.63
Tryptophan	0.24	0.25	0.27	0.28	0.30
Calcium	0.90	0.90	0.90	0.90	0.90
Available phosphorus	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20
ME (kcal/kg)	3,200	3,200	3,200	3,200	3,200

[1]Trace mineral premix added at this rate yields 149.6 mg of manganese, 125.1 mg of zinc, 16.5 mg of iron, 1.7 mg of copper, 1.05 mg of iodine, 0.25 mg of selenium, a minimum of 6.27 mg of calcium, and a maximum of 8.69 mg of calcium per kilogram of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[2]Vitamin premix added at this rate yields 11,023 IU of vitamin A, 3,858 IU of vitamin D_3, 46 IU of vitamin E, 0.0165 mg of B_{12}, 5.845 mg of riboflavin, 45.93 mg of niacin, 20.21 mg of D-pantothenic acid, 477.67 mg of choline, 1.47 mg of menadione, 1.75 mg of folic acid, 7.17 mg of pyroxidine, 2.94 mg of thiamine, and 0.55 mg of biotin per kilogram of diet. The carrier is ground rice hulls.

Experiment 3

Based on the results of the previous 2 experiments, experiment 3 investigated the effect of starter diet duration on broiler chick performance during an anticoccidial vaccination program with subsequent *Eimeria* challenge. The experimental design was a 3 × 2 × 2 factorial with the variables of starter period duration (13, 17, and 21 d), vaccination (vaccinated compared with nonmedicated nonvaccinated), and a mixed-species *Eimeria* challenge (challenged and nonchallenged). Each treatment was replicated in triplicate for a total of 36 pens. Each replicate contained 25 chicks placed, for a total of 900 chicks placed. Again, chicks were individually weighed, wing banded, and assigned to treatment groups by BW. Chicks assigned to the vaccinated treatment groups were vaccinated with Coccivac-B (Intervet/Schering Plough Animal Health) in a commercial spray cabinet according to the manufacturer's instructions. After spray vaccination, chicks were allowed to preen for 1 h before placement to allow for vaccine uptake. Pen weights and feed consumption were determined on d 13, 17, 21, and 27. The 22% starter diet used in the previous 3 experiments was fed to broilers for 13, 17, or 21 d depending on treatment. At the conclusion of the starter period, rations were switched to a grower diet formulated to meet the specifications of the high-nutrient-density diet specified in Leeson and Summers (2005), with the exception of the energy value being maintained at 3,200 kcal/kg. On d 21, broilers were challenged with a mixed-species challenge consisting of *E. acervulina* (1.25×10^6), *E. maxima* (4×10^5), and *E. tenella* (5×10^4) sporulated oocysts. On d 27 (6 d postchallenge), pen weights were taken, feed consumption was determined, and 10 broilers from each replicate pen were necropsied to determine the development of intestinal lesions (Johnson and Reid, 1970).

Statistical Analysis

Experiment 1. Data were analyzed using SPSS for Windows (SPSS, 2001) for all experiments. Statistical significance was determined by one-way ANOVA, and means were separated by Duncan's multiple range test. The threshold for statistical significance was $P \leq 0.05$.

Experiment 2. Body weight and feed conversion ratio for d 21 data were analyzed using one-way ANOVA because a significant interaction was present between protein level and vaccination. Significant differences were determined at $P \leq 0.05$, and means were separated using Duncan's multiple range test. Similarly, because of an interaction between challenge and vaccine, the data collected on d 27, including postchallenge BW gain, feed conversion ratio, mortality, and lesion scores, were analyzed using one-way ANOVA, with differences deemed significant at $P \leq 0.05$. Means were separated using Duncan's multiple range test. Postchallenge mortality was subjected to a square root arcsine transformation before analysis.

Table 2. Average BW (g) and mortality-corrected feed conversion ratio ± SE of broilers vaccinated with live oocysts[1] at d 21 when fed selected concentrations of protein (experiment 1)

Protein (%)	BW (g)	Feed:gain
20	585 ± 12^{c}	1.57 ± 0.03^{a}
21	665 ± 5^{b}	1.40 ± 0.02^{b}
22	679 ± 11^{b}	1.35 ± 0.01^{c}
23	689 ± 14^{ab}	1.34 ± 0.02^{c}
24	720 ± 9^{a}	1.27 ± 0.01^{d}

[a–d]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

Experiment 3. Prechallenge data were analyzed using 3 × 2 factorial ANOVA. Differences in main effects were deemed significant at $P \leq 0.05$, and means were separated using Duncan's multiple range test. Postchallenge data were analyzed using one-way ANOVA because a significant interaction was present between vaccine and challenge. Means were deemed significantly different at $P \leq 0.05$ and were separated using Duncan's multiple range test. Postchallenge mortality was analyzed in a similar fashion, following a square root arcsine transformation.

RESULTS

Experiment 1

As anticipated, average broiler BW at d 21 increased as protein level in the diet increased (Table 2). The 20% protein starter diet yielded lower ($P < 0.05$) average BW compared with all other treatments. The 24% protein diet yielded higher ($P < 0.05$) average BW compared with the 21 and 22% protein starter diets, whereas the 23% protein starter diet was similar. Feed conversion results yielded an inverse relationship with BW, with increasing protein level reducing mortality-corrected feed conversion of vaccinated broilers at 21 d of age. The 20% protein diet resulted in a higher ($P < 0.05$) mortality-corrected feed conversion ratio compared with those of all other treatments. Increasing the dietary protein concentration to 21% reduced ($P < 0.05$) the feed conversion ratio compared with 20% dietary protein. Further increases in protein level to 22 and 23% reduced ($P < 0.05$) the feed conversion ratios, whereas the 24% protein starter diet resulted in a lower ($P < 0.05$) mortality-corrected feed conversion ratio compared with all other protein levels. Average BW and mortality-corrected feed conversion ratio of vaccinated broilers fed 20, 22, and 24% dietary protein were significantly different from each other at 21 d of age. Therefore, these 3 protein levels were selected for use in experiment 2.

Experiment 2

Average BW of broilers at 21 d followed a trend similar to the observations reported in experiment 1, in which the increasing dietary protein level increased the average BW of broilers (Table 3). In nonvaccinated broilers, increases ($P < 0.05$) in the average BW were observed with each increase in dietary protein level. Body weights for vaccinated broilers followed a similar trend. The 20% protein level resulted in lower ($P < 0.05$) BW than did the 22 and 24% starter diets. Increases in BW resulting from increases in dietary protein from 22 to 24% were not observed in vaccinated broilers. Vaccinated broilers fed the lowest protein level in the starter diet were the lightest broilers compared with broilers in all other treatments. Vaccination of broilers reduced ($P < 0.05$) BW at the 20 and 24% protein levels compared with the BW of nonvaccinated broilers fed the same protein level. However, the vaccinated broilers fed 22% protein were similar to nonvaccinated broilers fed the same protein concentration.

Mortality-corrected feed conversion ratios at 21 d were similar to those in experiment 1, with increasing protein levels resulting in reduced feed conversion ratios for both vaccinated and nonvaccinated broilers (Table 3). Broilers fed the 20% protein diet yielded a higher ($P < 0.05$) feed conversion ratio compared with broilers fed the 22 and 24% protein diets regardless of vaccination. Vaccination resulted in an increase ($P < 0.05$) in feed conversion at the lowest protein level investigated. However, at the 2 highest protein levels fed, no differences were observed between vaccinated and nonvaccinated broilers with respect to feed conversion at each of the dietary protein levels.

Body weight gain and mortality-corrected feed conversion ratio during the challenge period (d 21 to 27) were similar for nonvaccinated, nonchallenged and vac-

Table 3. Average BW and mortality-corrected feed conversion ratio ± SE of nonvaccinated and vaccinated[1] broilers at d 21 when fed diets containing 3 different protein concentrations (experiment 2)

Protein (%)	Treatment	BW (g)	Feed:gain
20	Nonvaccinated	664 ± 18^{c}	1.40 ± 0.03^{b}
20	Vaccinated	579 ± 8^{d}	1.60 ± 0.02^{a}
22	Nonvaccinated	733 ± 33^{b}	1.30 ± 0.01^{cd}
22	Vaccinated	709 ± 14^{bc}	1.34 ± 0.02^{bc}
24	Nonvaccinated	808 ± 10^{a}	1.26 ± 0.01^{d}
24	Vaccinated	737 ± 17^{b}	1.31 ± 0.02^{cd}

[a–d]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

Table 4. Average BW gains, mortality-corrected feed conversion ratios (FCR), and mortality ± SE of nonvaccinated (NV) and vaccinated (V)[1] broilers 6 d after mixed-species *Eimeria* challenge at d 21, when fed diets containing 3 different protein concentrations (experiment 2)

Protein (%)	Vaccine	Challenge[2]	BW gain (g)	FCR d 21 to 27	FCR d 1 to 27	Postchallenge mortality[3]
20	NV	No	392 ± 7^{a}	1.59 ± 0.02^{d}	1.49 ± 0.03^{d}	0.07 ± 0.07^{bc}
20	V	No	377 ± 32^{a}	1.61 ± 0.05^{d}	1.60 ± 0.02^{c}	0.00 ± 0.00^{c}
22	NV	No	402 ± 8^{a}	1.39 ± 0.16^{d}	1.38 ± 0.01^{e}	0.00 ± 0.00^{c}
22	V	No	406 ± 16^{a}	1.52 ± 0.04^{d}	1.41 ± 0.02^{e}	0.00 ± 0.00^{c}
24	NV	No	415 ± 15^{a}	1.49 ± 0.02^{d}	1.35 ± 0.01^{e}	0.07 ± 0.07^{bc}
24	V	No	396 ± 12^{a}	1.49 ± 0.02^{d}	1.35 ± 0.01^{e}	0.00 ± 0.00^{c}
20	NV	Yes	63 ± 17^{d}	5.80 ± 0.46^{a}	1.78 ± 0.03^{a}	0.35 ± 0.09^{a}
20	V	Yes	236 ± 21^{c}	2.05 ± 0.09^{d}	1.74 ± 0.05^{a}	0.16 ± 0.08^{abc}
22	NV	Yes	96 ± 6^{d}	4.61 ± 0.22^{b}	1.67 ± 0.01^{b}	0.33 ± 0.03^{a}
22	V	Yes	278 ± 19^{bc}	1.89 ± 0.03^{d}	1.50 ± 0.01^{d}	0.07 ± 0.07^{bc}
24	NV	Yes	84 ± 28^{d}	3.91 ± 0.60^{c}	1.59 ± 0.05^{c}	0.23 ± 0.12^{ab}
24	V	Yes	316 ± 24^{b}	1.90 ± 0.03^{d}	1.50 ± 0.01^{d}	0.19 ± 0.10^{abc}

[a–e]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

[2]Mixed-species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

[3]Reported values are a result of a square root arcsine transformation of the observed mortality rates.

cinated, nonchallenged broilers at all protein levels (Table 4). In challenged broilers, vaccination increased ($P < 0.05$) BW gain compared with the BW of nonvaccinated broilers at all protein levels investigated. Nonvaccinated, challenged broilers had similar BW gains at all protein levels during the challenge period, which were lower ($P < 0.05$) than those of broilers in all other treatments. Within the vaccinated, challenged broilers, increased ($P < 0.05$) BW gains were observed in broilers fed the 24% protein diet compared with those fed the 20% protein diet, whereas the BW gain was intermediate in those fed the 22% protein diet. Mortality-corrected feed conversion ratios during the challenge period for vaccinated, challenged broilers were similar at all protein levels fed. In lieu of challenge, vaccinated broilers yielded feed conversion ratios similar to those of all the nonchallenged treatment groups. Challenge of the nonvaccinated broilers resulted in higher ($P < 0.05$) feed conversion ratios compared with those of all other treatment groups. In nonvaccinated, challenged broilers, postchallenge feed conversion ratios were decreased ($P < 0.05$) with each increase in dietary protein level. An increased protein concentration improved broiler performance during *Eimeria* challenge, with an increased BW gain in vaccinated broilers and a decreased feed conversion ratio in nonvaccinated broilers.

The cumulative mortality-corrected feed conversion ratios for d 1 to 27 were similar in nonvaccinated and vaccinated, nonchallenged broilers at the 22 and 24% protein levels. Nonchallenged broilers fed the 20% protein diet had a higher ($P < 0.05$) feed conversion ratio compared with those fed the 22 and 24% protein diets. Vaccination caused an increase ($P < 0.05$) in cumulative feed conversion at the 20% level in nonchallenged broilers but had no adverse effect in nonchallenged broilers fed the 22 and 24% protein diets. In challenged broilers, vaccination led to a decrease ($P < 0.05$) in the cumulative feed conversion ratio in broilers fed the 22 and 24% protein diets compared with nonvaccinated, challenged broilers, whereas the 20% protein diet was similar for both vaccinated and nonvaccinated, challenged broilers. Increased dietary protein led to a decreased ($P < 0.05$) cumulative feed conversion ratio in nonvaccinated, challenged broilers. Vaccination resulted in decreased ($P < 0.05$) mortality in challenged broilers fed the 22% protein diet compared with nonvaccinated, challenged broilers fed the 20 and 22% protein diets, whereas all other challenged groups had similar mortality rates.

Overall, lesion development was decreased in vaccinated, challenged broilers compared nonvaccinated, challenged broilers (Table 5). Lesions in the upper small intestine indicative of *E. acervulina* were minimal in all broilers. All vaccinated, challenged broilers had ($P < 0.05$) less lesion development in the upper small intestine compared with nonvaccinated, challenged broilers fed the 22 and 24% protein diets. Midintestinal lesion development associated with *E. maxima* was decreased ($P < 0.05$) in vaccinated, challenged broilers fed the 24% protein diet compared with nonvaccinated, challenged broilers fed the 24% protein diet. Lower intestinal lesion development associated with *E. tenella* challenge was decreased ($P < 0.05$) in vaccinated, challenged broilers compared with nonvaccinated, challenged broilers at all protein levels. Lower intestinal lesion scores were similar for all nonvaccinated, challenged broilers, whereas vaccinated, challenged broilers fed the 24% protein diet had significantly increased lower intestinal lesion scores compared with vaccinated, challenged broilers fed the 20 and 22% protein diets. An inverse relationship existed between mid and lower intestinal lesion development in vaccinated, challenged broilers fed diets with different concentrations of dietary protein. Midintestinal lesions decreased with increasing protein level, whereas

Table 5. Lesion scores of nonvaccinated (NV) and vaccinated (V)[1] broilers 6 d after mixed-species *Eimeria* challenge at d 21 of one-half the treatment groups fed diets containing 3 different protein concentrations (experiment 2)

Protein (%)	Vaccine	Challenge[2]	Upper	Mid	Lower
20	NV	No	0.26 ± 0.08^{bc}	0.40 ± 0.12^{e}	0.00 ± 0.00^{d}
20	V	No	0.00 ± 0.00^{d}	0.87 ± 0.25^{de}	0.23 ± 0.12^{d}
22	NV	No	0.00 ± 0.00^{d}	0.64 ± 0.16^{e}	0.04 ± 0.04^{d}
22	V	No	0.00 ± 0.00^{d}	0.87 ± 0.10^{de}	0.03 ± 0.03^{d}
24	NV	No	0.10 ± 0.05^{cd}	0.47 ± 0.12^{e}	0.00 ± 0.00^{d}
24	V	No	0.00 ± 0.00^{d}	0.60 ± 0.18^{e}	0.07 ± 0.04^{d}
20	NV	Yes	0.13 ± 0.08^{cd}	2.13 ± 0.26^{a}	2.17 ± 0.11^{a}
20	V	Yes	0.00 ± 0.00^{d}	1.90 ± 0.24^{ab}	0.60 ± 0.15^{c}
22	NV	Yes	0.33 ± 0.09^{b}	1.83 ± 0.22^{ab}	2.03 ± 0.11^{a}
22	V	Yes	0.03 ± 0.03^{d}	1.57 ± 0.19^{bc}	0.77 ± 0.17^{c}
24	NV	Yes	0.53 ± 0.15^{a}	1.93 ± 0.23^{ab}	2.03 ± 0.11^{a}
24	V	Yes	0.00 ± 0.00^{d}	1.20 ± 0.14^{cd}	1.40 ± 0.20^{b}

[a–e]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

[2]Mixed-species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

lower intestinal lesion development increased with increasing protein level. Lesions were observed in a small percentage of nonchallenged broilers. The lesions present in the nonvaccinated, nonchallenged broilers may be attributed to the close proximity with which the broilers were reared, whereas the lesions present in the vaccinated, nonchallenged broilers may be due to continued cycling of vaccine oocysts or the close proximity of rearing. Housing both challenged and nonchallenged broilers in close proximity to each other was essential to minimize environmental or pen-related effects on performance throughout the duration of the experiment.

Experiment 3

Experiment 3 investigated the effects of the duration of the starter phase on broiler performance during vaccination and subsequent challenge. Average BW for vaccinated and nonvaccinated broilers were similar ($P > 0.05$) at 13 d of age (Table 6). Vaccinated broilers had lower ($P < 0.05$) average BW compared with nonvaccinated broilers at 17 d of age. At 17 d of age, differences were not observed between broilers fed the starter diet for 13 d as opposed to 17 d. At 21 d of age, vaccinated broilers had lower ($P < 0.05$) average BW compared with nonvaccinated broilers, whereas the duration of the starter period had no effect on average BW. Mortality-corrected feed conversion ratios at 13 d were similar ($P > 0.05$) for vaccinated and nonvaccinated broilers (Table 7), and similar ($P > 0.05$) results were observed on 17 and 21 d. Vaccination had no adverse effects on feed conversion ratio during the first 21 d of age. An increased starter period duration resulted in decreased feed conversion ratios on d 21. Broilers switched to the grower diet on d 13 had an increased ($P < 0.05$) feed conversion compared with broilers switched to the grower diet on d 17 and 21. This was the only adverse effect associated with a shorter duration of the starter phase.

During the challenge period (21 to 27 d of age), BW gains were similar for all nonvaccinated and vaccinated, nonchallenged treatments. In challenged broilers, all vaccinated treatment groups gained more ($P < 0.05$) BW than did nonvaccinated broilers (Table 8). No effect of duration of the starter phase was observed on BW gain during the challenge period. Mortality-cor-

Table 6. Average BW ± SE of nonvaccinated and vaccinated[1] broilers fed a starter diet for 3 different durations (experiment 3)

	BW (g)		
Item	Nonvaccinated	Vaccinated	Main effect mean
d 1 to 13	373 ± 4	375 ± 3	
d 1 to 17			
13-d duration	562 ± 7	537 ± 10	549 ± 7
17-d duration	566 ± 5	551 ± 8	559 ± 5
Main effect mean	564 ± 4^{a}	546 ± 7^{b}	
d 1 to 21			
13-d duration	757 ± 16	715 ± 19	736 ± 14
17-d duration	767 ± 10	757 ± 14	762 ± 8
21-d duration	777 ± 6	739 ± 15	760 ± 18
Main effect mean	767 ± 6^{a}	737 ± 13^{b}	

[a,b]Main effect means with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

Table 7. Mortality-corrected feed conversion ratio (FCR) ± SE of nonvaccinated and vaccinated[1] broilers fed a starter diet for 3 different durations (experiment 3)

	FCR		
Item	Nonvaccinated	Vaccinated	Main effect mean
d 1 to 13	1.10 ± 0.01	1.10 ± 0.01	
d 1 to 17			
13-d duration	1.30 ± 0.01	1.32 ± 0.02	1.31 ± 0.02
17-d duration	1.28 ± 0.02	1.30 ± 0.02	1.29 ± 0.02
Main effect mean	1.29 ± 0.01	1.31 ± 0.02	
d 1 to 21			
13-d duration	1.40 ± 0.02	1.47 ± 0.04	1.44 ± 0.03[a]
17-d duration	1.36 ± 0.02	1.37 ± 0.01	1.37 ± 0.01[b]
21-d duration	1.34 ± 0.01	1.37 ± 0.02	1.35 ± 0.01[b]
Main effect mean	1.37 ± 0.01	1.41 ± 0.02	

[a,b]Main effect means with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

rected feed conversion ratios were similar for vaccinated and nonvaccinated, nonchallenged broilers during the challenge period. In challenged broilers, vaccination resulted in decreased ($P < 0.05$) feed conversion ratios compared with those of nonvaccinated broilers postchallenge. Cumulative feed conversion ratios (1 to 27 d) were similar for broilers in all nonchallenged treatments. All cumulative feed conversion ratios were higher ($P < 0.05$) for challenged broilers compared with nonchallenged broilers. The cumulative feed conversion ratios in vaccinated, challenged broilers fed the starter diet for 21 d were reduced ($P < 0.05$) compared with those of nonvaccinated, challenged broilers fed the starter diet for 13 and 17 d. Postchallenge mortality was higher ($P < 0.05$) in the nonvaccinated, challenged groups compared with all other treatment groups, whereas mortality for vaccinated, challenged broilers was similar to that of all nonchallenged broilers. An effect of starter phase duration on postchallenge mortality was not observed. Lesion development associated with the mixed-species challenge was unaffected by duration of the starter phase. However, lesion development was reduced ($P < 0.05$) in the upper and lower intestinal segments in vaccinated, challenged broilers compared with nonvaccinated, challenged broilers (Table 9). Although lesion development associated with the *E. maxima* challenge was numerically reduced in vaccinated, challenged broilers compared with nonvaccinated, challenged broilers, the lesion development of only broilers switched to the grower diet at 13 d of age reached the level of significance.

DISCUSSION

Increasing dietary protein concentration improved broiler performance at 21 d of age, as determined by BW and feed conversion, regardless of vaccination. This observation was expected because many reports have correlated the CP level of diets with broiler performance (Sterling et al., 2003; Vieira et al., 2004). The CP level of diets is of extreme importance because of the cost associated with increasing the protein level in

Table 8. Average BW gains, mortality-corrected feed conversion ratio (FCR), and mortality ± SE of nonvaccinated (NV) and vaccinated (V)[1] broilers 6 d after mixed-species *Eimeria* challenge at d 21, when fed a starter diet for 3 different durations (experiment 3)

				FCR		
Duration (d)	Vaccine	Challenge[2]	BW gain (g)	d 21 to 27	d 1 to 27	Postchallenge mortality[3]
13	NV	No	418 ± 63[a]	1.55 ± 0.19[b]	1.46 ± 0.07[c]	0.07 ± 0.07[b]
13	V	No	435 ± 25[a]	1.34 ± 0.07[b]	1.41 ± 0.05[c]	0.00 ± 0.00[b]
17	NV	No	448 ± 32[a]	1.44 ± 0.05[b]	1.41 ± 0.01[c]	0.00 ± 0.00[b]
17	V	No	402 ± 24[a]	1.55 ± 0.02[b]	1.44 ± 0.01[c]	0.00 ± 0.00[b]
21	NV	No	409 ± 48[a]	1.72 ± 0.24[b]	1.46 ± 0.06[c]	0.00 ± 0.00[b]
21	V	No	441 ± 15[a]	1.47 ± 0.04[b]	1.43 ± 0.02[c]	0.00 ± 0.00[b]
13	NV	Yes	81 ± 11[c]	5.77 ± 0.50[a]	1.73 ± 0.02[a]	0.49 ± 0.04[a]
13	V	Yes	242 ± 14[b]	2.01 ± 0.22[b]	1.62 ± 0.03[ab]	0.14 ± 0.07[b]
17	NV	Yes	102 ± 2[c]	5.43 ± 0.32[a]	1.73 ± 0.01[a]	0.49 ± 0.02[a]
17	V	Yes	210 ± 11[b]	2.07 ± 0.14[b]	1.65 ± 0.01[ab]	0.14 ± 0.07[b]
21	NV	Yes	113 ± 36[c]	4.84 ± 0.58[a]	1.67 ± 0.03[ab]	0.36 ± 0.04[a]
21	V	Yes	271 ± 22[b]	2.27 ± 0.12[b]	1.59 ± 0.05[b]	0.08 ± 0.08[b]

[a–c]Means with different superscripts within columns differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

[2]Mixed-species challenge contained *Eimeria acervulina* (1.25×10^6), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (5×10^4) sporulated oocysts.

[3]Reported values are a result of a square root arcsine transformation of the observed mortality rates.

Table 9. Intestinal lesion scores of nonvaccinated (NV) and vaccinated (V)[1] broilers 6 d after mixed-species *Eimeria* challenge at d 21, when fed a starter diet for 3 different durations (experiment 3)

Duration (d)	Vaccine	Challenge[2]	Upper	Mid	Lower
13	NV	No	0.03 ± 0.03^{c}	0.13 ± 0.06^{d}	0.00 ± 0.00^{d}
13	V	No	0.00 ± 0.00^{c}	0.60 ± 0.11^{c}	0.10 ± 0.06^{d}
17	NV	No	0.00 ± 0.00^{c}	0.07 ± 0.05^{d}	0.00 ± 0.00^{d}
17	V	No	0.03 ± 0.03^{c}	0.63 ± 0.10^{c}	0.03 ± 0.03^{d}
21	NV	No	0.00 ± 0.00^{c}	0.13 ± 0.06^{d}	0.00 ± 0.00^{d}
21	V	No	0.00 ± 0.00^{c}	0.47 ± 0.08^{cd}	0.03 ± 0.02^{d}
13	NV	Yes	1.40 ± 0.18^{ab}	2.30 ± 0.15^{a}	2.10 ± 0.15^{a}
13	V	Yes	0.23 ± 0.09^{c}	1.70 ± 0.20^{b}	0.63 ± 0.15^{c}
17	NV	Yes	1.47 ± 0.21^{a}	2.37 ± 0.17^{a}	2.23 ± 0.16^{a}
17	V	Yes	0.13 ± 0.08^{c}	2.07 ± 0.20^{ab}	1.13 ± 0.21^{b}
21	NV	Yes	1.13 ± 0.13^{b}	2.13 ± 0.15^{a}	2.30 ± 0.14^{a}
21	V	Yes	0.10 ± 0.06^{c}	2.07 ± 0.18^{ab}	1.13 ± 0.17^{b}

[a–d]Means within columns with different superscripts differ significantly ($P < 0.05$).

[1]Coccivac-B (Intervet/Schering Plough Animal Health, Summit, NJ).

[2]Mixed-species challenge contained *Eimeria acervulina* (1.25×10^{6}), *Eimeria maxima* (4×10^{5}), and *Eimeria tenella* (5×10^{4}) sporulated oocysts.

diets, which leads to effects on costs as well as on revenues during the production of broiler meat (Eits et al., 2005). In experiment 1, a linear relationship was observed with respect to BW and feed conversion associated with an increased CP level to 21 d of age in vaccinated broilers. Broilers fed 20, 22, or 24% protein in the starter diet to 21 d of age differed significantly in their performance characteristics. Therefore, these protein levels were selected for use in subsequent experiments to compare growth characteristics with those of nonvaccinated broilers during challenge with field strain *Eimeria*.

Nonvaccinated broilers had similar patterns of performance as vaccinated broilers, with an improvement in performance characteristics related to an increased protein level of the diet. Vaccination tended to reduce BW and increase feed conversion compared with nonvaccination before challenge. However, in experiment 2, in which an interaction was observed and data were analyzed by one-way ANOVA, vaccinated broilers fed 22% protein had growth characteristics similar to those of nonvaccinated broilers fed the 22% protein starter diet. Reduced BW and increased feed conversion ratio during the early stages of growth attributable to vaccination have been reported by other investigators (Danforth, 1998; Williams, 2002). However, other published reports have indicated that compensatory BW gain in vaccinated broilers during subsequent dietary periods results in similar, if not improved, performance characteristics at the completion of grow-out (Danforth, 1998; Williams et al., 1999; Williams, 2002; Williams and Gobbi, 2002; Lee et al., 2009).

During the 6-d challenge periods within this study, which began on d 21, vaccination did not adversely affect BW gain and the feed conversion ratio. Similarly, increasing dietary protein had no effect on performance parameters during this 6-d period. Over the duration of the experiment, the feed conversion ratio of nonchallenged broilers was unaffected by vaccination with respect to the 22 and 24% protein diets. Regardless of immunization status, nonchallenged broilers fed the 20% protein diet had increased feed conversion ratios compared with broilers fed the 22 and 24% protein diets. However, vaccination significantly increased the feed conversion ratios of broilers fed the 20% protein diet compared with nonvaccinated broilers fed the 20% protein diet in experiment 2. Diets containing the lowest protein level in these experiments tended to have a larger effect on performance characteristics because of vaccination. The highest dietary protein (24%) did not translate to the highest level of performance in vaccinated and challenged chickens because the 22% protein level was often indistinguishable from the 24% level. In experiment 2, performance values of chickens fed 22% dietary protein did not differ between the vaccinated and nonvaccinated groups before or during challenge.

It has been widely reported (Brake et al., 1997; Weber and Evans, 2003; Williams, 2003; Shirley et al., 2005) that the generation of immunity through vaccination improves the performance of broilers during *Eimeria* challenge, as was evident by the significantly increased BW gain, reduced feed conversion ratio, reduced lesion development, and, in some cases, reduced mortality postchallenge compared with those of nonvaccinated broilers. Improved growth characteristics during the challenge period in vaccinated broilers led to significantly improved cumulative feed conversion ratios (d 1 to 27) in broilers fed the 2 higher protein diets in experiment 2.

In experiment 2, increasing the protein level tended to reduce lesion development associated with *E. maxima* while tending to increase lesion development associated with *E. tenella* in vaccinated chickens. These data tend to indicate an inverse relationship in lesion development associated with *E. maxima* and *E. tenella* during a mixed-species challenge in vaccinated broilers fed increasing levels of dietary protein. Further investigation into the relationship of dietary protein and lesion development is needed before additional conclusions can be drawn.

The results obtained from the first 2 experiments indicate that a dietary protein inclusion rate of 22% in the starter diet allowed for similar growth characteristics in vaccinated and nonvaccinated broilers. Diets in these 2 experiments were not changed according to broiler age, and the starter diet was fed to either d 21 or 27, which is not applicable in modern poultry production. With this in mind, experiment 3 focused on the relationship between vaccination and the duration of the starter period. Vaccination had no effect on BW and feed conversion ratio on d 13 of age. Growth depression associated with vaccination was observed at 17 d of age, which corresponds with the second cycling of vaccine oocysts in the rearing environment, and it continued to d 21 (Williams, 2002). Growth depression was not observed in vaccinated broilers fed 22% protein in experiment 2. It is interesting that through d 21, the feed conversion ratio was unaffected by vaccination, with vaccinated and nonvaccinated broilers having similar feed conversion ratios. Body weights were unaffected by broiler age when broilers were changed to a grower diet, although those changed to a grower diet on d 13 had increased feed conversion compared with those changed on d 17 or 21 prechallenge. The day of change from the starter to grower diet had no effect on BW gain, feed conversion ratio, or mortality during the challenge period although vaccination did improve all these characteristics in challenged broilers. Nonvaccinated, challenged broilers had a significantly higher mortality rate compared with vaccinated, challenged broilers, a finding supported by the observations of Williams et al. (1999).

Within these experiments, broilers immunized with Coccivac-B (Intervet/Schering Plough Animal Health) had significant protection against lesion development associated with *E. acervulina* and *E. tenella.* Reductions in lesion scores in the midintestinal segments were observed, but not to the same extent as lesions present in the other 2 sites of infection. This can most likely be attributed to the immunogenic variability observed with *E. maxima.* To gain significant immunization with the use of a commercially available live oocyst vaccine, Danforth et al. (1997) altered the vaccine used by adding *E. maxima* strains isolated locally.

Data from this series of experiments indicate that vaccination with a live oocyst vaccine is an effective tool for generating immunity to field strain *Eimeria* challenge. Vaccination resulted in improved growth parameters in immunized compared with nonimmunized broilers after a challenge. Further observations indicated that dietary protein level is an important factor to consider when using a vaccination program for the prevention of coccidiosis to maximize growth characteristics, regardless of the duration of the starter period. Increasing dietary protein concentrations may be one management consideration that might reduce or eliminate the adverse effects on broiler performance resulting from vaccination during the starter period.

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