Effects of dietary enzymes on performance and intestinal goblet cell number of broilers exposed to a live coccidia oocyst vaccine

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ABSTRACT An experiment was conducted to evaluate the effects of dietary enzymes on performance, tibia ash, and intestinal goblet cells of broilers administered a live coccidia oocyst vaccine (Coccivac B, Schering Plough, Kenilworth, NJ). Cobb 500 straight-run broilers were obtained and one-half of the chicks were sprayed with the live coccidia oocyst vaccine. Chicks were weighed and placed in battery brooders with respect to nonvaccinated or vaccinated group according to dietary treatment. The 8 dietary treatments were a positive control (0.90% Ca and 0.45% available P), a negative control (NC; 0.80% Ca and 0.35% available P), NC + phytase (PHY), NC + protease (PRO), NC + xylanase (XYL), NC + PHY+ PRO, NC + PHY + XYL, and NC + PHY + PRO + XYL. A diet × vaccination interaction ($P > 0.05$) was not observed for feed intake or BW gain. Feed conversion ratio was improved ($P \leq 0.05$) in birds fed NC + PHY + XYL compared with NC. Vaccination reduced ($P \leq 0.05$) feed intake and BW gain from d 0 to 18. Tibia ash was reduced ($P \leq 0.05$) in the NC and PRO or XYL diets. Vaccination increased goblet cell numbers in the duodenum of birds fed XYL, whereas no differences were found in goblet cell numbers between nonvaccinated and vaccinated birds in other dietary treatments, which resulted in a diet × vaccination interaction ($P \leq 0.05$). Protease decreased and NC + PHY+ PRO increased goblet cells in the jejunum at d 7, which resulted in a diet × vaccination interaction ($P \leq 0.05$). At d 18, NC + PHY + XYL was the only diet in which vaccination decreased goblet cells in the jejunum, resulting in a diet × vaccination interaction ($P \leq 0.05$). The data indicate that NC + PHY + XYL improved the feed conversion ratio in broilers fed corn-soybean meal diets. The vaccination × dietary enzyme interaction altered the number of goblet cells in the small intestine. Dietary enzyme supplementation did not alleviate reductions in growth performance associated with the use of a live coccidia oocyst vaccine.

Key words: phytase, protease, xylanase, goblet cell, coccidia

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INTRODUCTION

Studies evaluating exogenous enzymes and enzyme cocktails in broiler diets have been published extensively in the last 20 yr. Enzymes are known to maintain broiler performance and improve the feed conversion ratio (**FCR**) in nutritionally marginal diets, in addition to reducing the antinutrient effects of certain dietary ingredients and improving nutrient availability (Bedford, 2000). More recently, researchers have evaluated the effects of dietary enzymes and enzyme cocktails on immune function and pathogen resistance. Watson et al. (2005) determined that phytase improved growth performance in *Eimeria acervulina*-challenged chicks. However, noninfected chicks were more efficient and had a larger tibia ash percentage than infected chicks. Phytase indirectly increased the intestinal absorption capacity of D-xylose (Mansoori et al., 2010) and protease increased BW gain (**BWG**) and small intestine mucus layer thickness (Peek et al., 2009) in broiler chicks orally gavaged with *Eimeria* spp. oocysts. Parker et al. (2007) determined that an enzyme cocktail containing amylase, protease, and xylanase altered microbial profiles in coccidia-vaccinated chicks but that it had no effect on ileal amino acid digestibility or growth performance.

Eimeria spp. parasites invade the chicken intestinal lumen, causing the disease coccidiosis. Coccidiosis results in reduced feed intake (**FI**) and BWG (Turk, 1972; Williams, 2002) and malabsorption of nutrients (Turk, 1972; Persia et al., 2006), possibly because of reduced

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brush border enzyme activities (Adams et al., 1996) and disruption of intestinal integrity (Turk, 1972; Assis et al., 2010). In addition, inoculation with *E. acervulina* and *Eimeria maxima* oocysts increased the size and number of goblet cells along ileal crypts in broilers (Collier et al., 2008). The epithelial cells of the gastrointestinal tract are covered by a protective mucus layer composed of high molecular weight glycoproteins called mucins (Pearson and Brownlee, 2005). Mucins are the first line of defense against intestinal pathogens and act to protect the epithelium within the intestinal lumen (Montagne et al., 2004). Integrity of the protective mucus layer is maintained by synthesis and secretion of mucins from goblet cells (Montagne et al., 2004).

Dietary enzymes have been reported to alter goblet cell numbers in the intestine of healthy laying hens (Yaghobfar et al., 2007) and broilers (Wu et al., 2004). However, other authors reported dietary enzyme supplementation had no effect on broiler small intestinal goblet cells (Yaghobfar et al., 2007).

Although the effects of enzyme supplementation or coccidia challenge on small intestine goblet cells have been reported, published data are lacking on the combined effect of enzyme supplementation and a live coccidia vaccine on this cell population. The aim of this study was to determine the influence of single dietary enzymes (phytase, xylanase, protease) or their combined use on performance, bone ash, and number of goblet cells in the small intestine of broilers fed reduced Ca and P corn-soybean meal diets and exposed to a mixed *Eimeria* spp. live coccidia oocyst vaccine.

MATERIALS AND METHODS

Animals and Husbandry

Straight-run Cobb 500 broilers (1,728) were obtained from a commercial hatchery at day of hatch and transported to the poultry research farm at Virginia Tech. On arrival, one-half of the chicks (864) were sprayed with a commercially available live coccidia oocyst vaccine (Coccivac B, Schering Plough, Kenilworth, NJ) according to the manufacturer's recommendations. Chicks were then randomly selected, weighed, and placed in Petersime battery brooders by nonvaccinated or vaccinated group. Nonvaccinated birds were placed in batteries separate from vaccinated birds in the same environmentally controlled room. Throughout the trial, care was taken to minimize cross-contamination of the oocysts via handling of nonvaccinated birds, feed, water, and excreta before handling the vaccinated birds. No evidence of gross lesions caused by *Eimeria* infection was present in the nonchallenged birds selected for sampling, and no *Eimeria* infection was identified during the morphological evaluation of intestinal tissues. Birds were maintained on a lighting program of 24L:0D on d 0, 22L:2D from d 1 to 7, and 18L:6D from d 7 to 18.

Diets and Enzymes

All diets were fed in mash form and formulated on a corn-soybean meal basis according to Cobb 500 requirements (Cobb 500 Broiler Performance and Nutrition Supplement, Cobb-Vantress Inc., Siloam Springs, AR), with the exception of Ca and available P in the negative control (**NC**; Table 1), which was reduced to account for the improvements associated with dietary phytase supplementation. Dietary treatments consisted of a positive control (**PC**; 0.90% Ca and 0.45% available P), NC (0.80% Ca and 0.35% available P), NC + phytase (**PHY**), NC + protease (**PRO**), NC + xylanase (**XYL**), NC + PHY + PRO, NC + PHY + XYL, and NC + PHY + PRO + XYL. An NC basal diet was mixed as 1 large batch to provide the base diet and then subdivided into 7 parts for the addition of enzymes to constitute other dietary treatments. The 7 experimental diets were made by adding phytase, protease, and xylanase. Sand was added to the NC diet in place of supplemental enzymes. The NC base diet and PC diet were analyzed (A and L Eastern Laboratories, Richmond, VA) according to the methods of the Association of Official Analytical Chemists (1995) for moisture (930.15) and CP (990.03). Calcium and P analyses were performed using the US Environmental Protection Agency (1996) solid waste method SW846-6010B. Nonvaccinated and vaccinated birds were allowed ad libitum access to 1 of the 8 dietary treatments from d 0 to 18. This resulted in a total of 16 treatments, with 9 replicate cages of 12 birds per cage from d 0 to 7 and 6 birds per cage from d 7 to 18.

The enzymes used in the experiment were supplied by Danisco Animal Nutrition (St. Louis, MO). The phytase used was an *Escherichia coli* 6-phytase expressed in *Schizosaccharomyces pombe* and formulated to provide 1,000 U/kg of diet (Phyzyme XP, Danisco Animal Nutrition). The protease used was extracted from *Bacillus subtilis* and formulated to provide 8,000 U/kg of diet (Subtilsin Protease, Danisco Animal Nutrition), and the xylanase was a *Trichoderma reesei* endo-1,4-β-xylanase formulated to provide 1,200 U/kg of diet (Porzyme Xylanase, Danisco Animal Nutrition). Phytase activity (1 phytase unit is defined as the amount of enzyme required to release 1 μmol of inorganic P/min from sodium phytate at 37°C) in the experimental diets was conducted (Danisco Animal Nutrition) according to the modified method of Engelen et al. (2001). Xylanase activity [1 xylanase unit is defined as the amount of enzyme that liberates 0.5 μmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt xylan substrate at pH 5.3 and 50°C in 1 min] and protease activity [1 protease unit is defined as the amount of enzyme that liberates 1 μmol of phenolic compound (tyrosine equivalents) from a casein substrate/min at pH 7.5 and 40°C] in the experimental diets were measured using modified methods based on the Megazyme (Megazyme International Ire-

land Ltd., Bray, Ireland) xylanase and protease assay kits. Diets were formulated to contain 0.3% titanium oxide as an indigestible marker. All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee.

Sample Collection

Birds were weighed by cage before placement (d 0) and on d 7 and 18 to measure mean BW and calculate mean BWG for d 0 to 7 and cumulatively (d 0 to 18). Feed intake and FCR were also measured for d 0 to 7 and cumulatively. Room temperature and mortality were recorded daily. Any birds removed for sampling or mortality were weighed, and FI and FCR were adjusted according to the number of bird days. This was defined as the number of birds alive in each cage multiplied by the number of days without incidence of mortality. Birds sampled were stunned by exposure to CO_2 gas for approximately 30 s and killed by cervical dislocation for collection of tissues for goblet cell counts and bone ash.

On d 7 and 18, one bird of average BW/cage (9 birds/diet per treatment) was killed to obtain tissues from the duodenum, jejunum, and ileum for determination of goblet cell numbers. Intestinal segments (approximately 2 to 3 cm) were obtained from the duodenum (proximal to the duodenal loop) and from the middle of the jejunum (defined as the intestinal section distal to the duodenal loop and proximal to Meckel's diverticulum) and ileum (defined as the intestinal section beginning at Meckel's diverticulum and ending at the ileocecal junction). The collected tissues were gently flushed with cold PBS to remove luminal contents and placed in 10% neutral buffered formalin until further processing. Each fixed intestinal segment was cut into five 5-mm sections and embedded in paraffin. Embedded samples were cut at 5 μm and mounted onto slides. Slides were stained using periodic acid-Schiff reagent and Alcian blue and were examined by light microscopy (Olympus Polaroid DMC-IE Camera, Polaroid Corp., Waltham, MA). Measurements were made using Sigma Scan Pro 5 software (SPSS Inc., Chicago, IL) and digitized using Image Pro Plus (Media Cybernetics, Silver Spring, MD). The number of goblet cells per villus was counted and the villus area was obtained from 4 villi/3 tissues per slide. The 12 measurements were then used to obtain a mean number of goblet cells per bird. The goblet cell number per villus area was calculated and normalized using the natural log.

Left tibias were obtained from 4 birds/cage (36 birds/diet per treatment) and pooled for determination of bone ash on d 7 and 18. Tibias were stripped of adhering tissues, wrapped in cheesecloth, and dried overnight at 100°C. Fat was extracted from the tibias using a Soxhlet apparatus and 100% ethyl ether according to modified methods of Watson et al. (2006). Fat-extracted tibias were then dried for 24 h at 100°C and ashed in a muffle furnace for 24 h at 600°C to determine bone ash.

Statistical Analysis

The data were subjected to ANOVA using the PROC MIXED procedure for a completely randomized design (SAS Institute, Cary, NC). Percentage of mortality data were arcsine transformed before analysis. Cage served as the experimental unit for FI, BWG, FCR, percentage of mortality, and tibia ash. Bird served as the experimental unit for goblet cell counts. The statistical model included diet, vaccination, and the interaction of diet × vaccination. When means were significant, differences were separated using Tukey's honestly sig-

Table 1. Composition and nutrient content of basal diets[1]

Item	Positive control (%)	Negative control (%)
Ingredient		
Corn	53.58	54.75
Soybean meal, 45%	33.13	32.89
Dried distillers grains with solubles	5.00	5.00
Poultry fat	3.93	3.52
Dicalcium phosphate[2]	1.65	1.11
Limestone[3]	1.14	1.16
Salt	0.48	0.48
Titanium oxide	0.30	0.30
DL-Methionine	0.25	0.25
L-Lysine hydrochloride	0.19	0.20
Trace mineral premix[4]	0.10	0.10
Vitamin premix[5]	0.10	0.10
Choline chloride	0.07	0.07
Sand	0.06	0.06
Calculated composition		
DM	88.12	88.00
CP	21.50	21.50
Lysine	1.30	1.30
TSAA	0.98	0.98
Threonine	0.82	0.82
Ca	0.90	0.80
Total P	0.70	0.60
Available P	0.45	0.35
Na	0.21	0.21
Nutrient composition		
ME (kcal/kg)	3,025	3,025
Analyzed composition (as-is basis)[6]		
DM	88.84	90.83
CP	20.47	19.91
Ca	1.02	0.91
Total P	0.67	0.62

[1]Diets were fed in mash form from d 0 to 18. Negative control diets were supplemented with an *Escherichia coli*-derived phytase expressed in *Schizosaccharomyces pombe*, *Bacillus subtilis* protease, and *Trichoderma longibrachiatum* endo-1,4-β-xylanase.

[2]Dicalcium phosphate supplied 18.5% P and 22% Ca.

[3]Limestone supplied 38% Ca.

[4]Supplied per kilogram of diet: iron (ferrous sulfate), 40 mg; manganese (manganese sulfate and manganous oxide), 120 mg; zinc (zinc oxide), 210 mg; cobalt (cobalt carbonate), 2.2 mg; iodine (calcium iodate), 132 mg.

[5]Supplied per kilogram of diet: vitamin A, 8,818 IU; vitamin D_3, 2,646 ICU; vitamin E, 22 IU; vitamin B_{12}, 26 μg; riboflavin, 8.8 mg; niacin, 88 mg; D-pantothenic acid, 22 mg; vitamin K, 2.6 mg; folic acid, 2.2 mg; vitamin B_6, 4.3 mg; thiamine, 3.7 mg; D-biotin, 220 μg.

[6]The analyzed values represent the mean of duplicate samples per analysis.

Table 2. Recovery of enzyme activity in experimental diets[1]

Diet (U/kg)	Determined phytase activity[2]	Determined protease activity[3]	Determined xylanase activity[4]
Positive control (PC)	60	<100	430
Negative control (NC)	<50	—	—
NC + phytase (PHY; 1,000 U)	1,222	—	—
NC + protease (PRO; 8,000 U)	—	6,759	—
NC + xylanase (XYL; 1,200 U)	—	—	1,787
NC + PHY + PRO	1,272	6,679	—
NC + PHY + XYL	955	—	1,079
NC + PHY + PRO + XYL	1,156	5,424	2,178

[1]The determined enzyme activity values represent the means of duplicate samples per analysis.

[2]One unit of phytase activity (FTU) is defined as the quantity of enzyme that liberates 1 μmol of inorganic P per minute from sodium phytate at pH 5.5 and 37°C.

[3]One unit is the amount of enzyme that liberates 1 μmol of phenolic compound (tyrosine equivalents) from a casein substrate per minute at pH 7.5 and 40°C.

[4]One unit is the amount of enzyme that liberates 0.5 μmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt xylan substrate at pH 5.3 and 50°C in 1 min.

nificant difference test. Statistical significance was accepted at $P \leq 0.05$.

RESULTS

The analyzed dietary Ca levels were higher than formulated (Table 1), but a 0.1% reduction in Ca was observed in the NC diets compared with the PC diet. Phosphorus was reduced in the NC basal diet, but by approximately 0.05% rather than the formulated 0.1%. Dietary analysis revealed that the recovery of enzymes was generally higher than expected but was in agreement with formulated values when mixing and assay errors were considered (Table 2). A small amount of xylanase was detected in the PC, which is common and may be associated with endogenous levels of the enzyme in plant ingredients or the result of assay variation. The xylanase activity in the other diets varied or was negligible, which may suggest that sampling or assay variation was quite high.

Mortality was low (<2%) throughout the experiment, and no differences were associated with diet or vaccination (data not shown). No dietary and vaccination interaction was found for FI, BWG, or FCR ($P > 0.05$); therefore, only the main effects for cumulative (d 0 to 18) performance data are presented (Table 3). Combined PHY and XYL supplementation improved ($P \leq 0.05$) FCR compared with the NC from d 0 to 18. All other dietary treatments were similar to each other. Vaccination with live coccidia oocysts reduced ($P \leq 0.05$) FI and BWG but had no effect ($P > 0.05$) on FCR (Table 3). Although no interactions were found between diet and vaccination for tibia ash percentage ($P > 0.05$; Table 4), a main effect of diet was found.

Table 3. Main effects of dietary enzyme supplementation or coccidia vaccination on the performance of broilers fed corn-soybean meal starter diets (0 to 18 d posthatch)[1]

Main effect	Feed intake (g)	BWG[2] (g)	Feed:gain (g/g)
Diet			
Positive control (PC)	838.9	633.8	1.324^{ab}
Negative control (NC)	830.0	620.7	1.338^{a}
NC + phytase (PHY; 1,000 U)	839.7	639.9	1.313^{ab}
NC + protease (PRO; 8,200 U)	821.4	615.6	1.335^{ab}
NC + xylanase (XYL; 1,200 U)	827.0	629.1	1.316^{ab}
NC + PHY + PRO	819.0	625.8	1.311^{ab}
NC + PHY + XYL	828.6	634.6	1.306^{b}
NC + PHY + PRO + XYL	831.1	630.3	1.319^{ab}
Pooled SEM	8.030		
Vaccination			
Nonvaccinated	836.4^{a}	635.6^{a}	1.317
Vaccinated	822.5^{b}	621.8^{b}	1.324
Pooled SEM	4.016		
P-value			
Diet	0.7765	0.4719	0.0442
Vaccination	0.0478	0.0169	0.1977
Diet × vaccination	0.3288	0.1569	0.3916

[a,b]Means in a column not sharing a common superscript are different ($P \leq 0.05$).

[1]Each mean represents an average of 18 replicate cages/diet and 72 replicate cages/vaccination treatment (12 birds/cage from d 0 to 7 and 6 birds/cage from d 7 to 18).

[2]BW gain.

Tibia ash on d 7 was reduced ($P \leq 0.05$) with the NC diet and the NC diets supplemented with PRO or XYL compared with all other treatments. On d 18, tibia ash percentage was lower with the NC + XYL diet compared with the NC + PHY + XYL or NC + PHY diets. Vaccination did not affect ($P > 0.05$) tibia ash at d 7 or 18.

Diet and vaccination had no effect ($P > 0.05$) on the number of goblet cells in the duodenum at d 7 (Table 5). On d 18 in the duodenum, vaccinated birds fed NC + XYL had significantly more goblet cells than nonvaccinated birds on this same diet. However, in all other dietary treatment groups, no difference was found in goblet cells between nonvaccinated and vaccinated birds, which resulted in a significant diet × vaccination interaction. Vaccination reduced the number of goblet cells in the jejunum of birds fed the PRO diet at d 7 (13%) as compared with nonvaccinated birds, but vaccination increased the number of goblet cells in the jejunum of birds fed NC diets supplemented with PHY + PRO (15%). This resulted in a significant ($P \leq 0.05$) interaction of diet and vaccination on the number of goblet cells in the jejunum at d 7. On d 18, vaccination and PHY + XYL supplementation reduced the number of goblet cells in the jejunum of vaccinated birds compared with this diet effect in nonvaccinated birds. No other effects of diet or vaccination were found, which resulted in a significant ($P \leq 0.05$) interaction of diet and vaccination on the number of goblet cells in the jejunum at d 18. Diet and vaccination did not have an effect ($P > 0.05$) on the number of goblet cells in the ileum on d 7 or 18 (data not shown).

DISCUSSION

In the current experiment, differences in Ca and P between the PC and NC basal diets were approximately 0.1 and 0.05%, respectively. Dietary P may not have been limiting to the bird regarding performance and may explain the similar FI and BWG between birds fed the NC and PC diets. Yan et al. (2006) determined that, in the absence of phytase, 0.31% nonphytate P and 0.90% Ca were required for maximum BW. Several authors have reported no effects (Pinheiro et al., 2004; Olukosi et al., 2007) or beneficial effects (Cowieson and Ravindran, 2008; Francesch and Geraert, 2009) on growth performance of broilers fed corn- and soybean meal-based diets supplemented with enzyme cocktails containing phytase, xylanase, protease, and amylase.

The combined supplementation of phytase and xylanase improved broiler FCR compared with broilers fed the NC diet. This has been reported previously in wheat-based diets (Selle et al., 2003) and corn- and soy-based diets (Francesch and Geraert, 2009). Supplementation of xylanase in corn-soybean meal diets may hydrolyze arabinoxylans and release cell wall-encapsulated starch or protein. Phytase may reduce the antinutrient effects of phytate, and in conjunction with the benefits of xylanase, may result in an improvement in net energy and feed efficiency (Cowieson, 2005).

In this study, the reduced total P and Ca in the NC diet was enough to result in a difference in tibia ash in 7-d-old broilers fed diets supplemented with phytase compared with broilers fed the basal NC diet or NC diet supplemented with protease or xylanase alone. Similar to published reports, phytase supplementation alone or in combination with the other enzymes improved P availability by hydrolyzing phytate and increasing bone ash (Onyango et al., 2005; Yan et al., 2006; Francesch and Geraert, 2009) compared with protease and xylanase alone. The effects of dietary enzyme supplementation on bone ash percentage at d 18 were less consistent, which may be the result of an increase in FI or a reduced Ca and P requirement as the bird ages.

Coccidia vaccination with the mixed-species oocyst population had no effect on tibia ash. In contrast, infection by challenge with *E. acervulina* reduced tibia ash percentage in chicks (Ward et al., 1993; Watson et al., 2005) but increased tibia Mg, Cu, Fe, Pb, Mn, and Al concentrations (Ward et al., 1993). The influence of coccidiosis on mineral absorption and metabolism may be related to the severity of the challenge or the species of *Eimeria*. Coccivac B uses a low dose of live oocysts from 3 of the most common species of *Eimeria* known to infect poultry (*E. acervulina*, *E. maxima*, and *Eimeria tenella*). Induction of a protective immune response requires recycling of the oocysts through a fecal-oral route. In the current experiment, chicks were housed on raised wire floors and exposed to a mild infection with the live vaccine on the day of hatch. Therefore, expo-

Table 4. Main effects of dietary enzyme supplementation or coccidia vaccination on the tibia ash percentage of broilers fed corn-soybean meal starter diets[1]

	Tibia ash (%)	
Main effect	Day 7	Day 18
Diet		
Positive control (PC)	47.72[a]	51.36[abc]
Negative control (NC)	46.17[b]	50.88[bc]
NC + phytase (PHY; 1,000 U)	48.03[a]	51.51[ab]
NC + protease (PRO; 8,200 U)	46.26[b]	50.85[bc]
NC + xylanase (XYL; 1,200 U)	46.17[b]	50.79[c]
NC + PHY + PRO	48.02[a]	51.25[abc]
NC + PHY + XYL	47.92[a]	51.69[a]
NC + PHY + PRO + XYL	47.71[a]	51.03[abc]
Pooled SEM	0.2557	0.1532
Vaccination		
Nonvaccinated	47.17	51.10
Vaccinated	47.33	51.24
Pooled SEM	0.1278	0.0766
P-value		
Diet	0.0001	0.0001
Vaccination	0.4001	0.2079
Diet × vaccination	0.4714	0.1519

[a–c]Means in a column not sharing a common superscript are different ($P \leq 0.05$).

[1]Each mean represents an average of 18 replicate cages/diet and 72 replicate cages/vaccination treatment (4 birds/cage).

Table 5. Influence of dietary enzyme supplementation and coccidia vaccination on the number of small intestinal goblet cells in broiler chicks fed corn-soybean meal diets[1]

	Duodenum		Jejunum	
Diet × vaccination	Day 7	Day 18	Day 7	Day 18
Diet				
Positive control (PC)				
−[2]	6.25	6.38^{abcd}	6.89^{abc}	6.86^{abc}
+[3]	6.34	6.59^{ab}	6.59^{cd}	6.79^{abc}
Negative control (NC)				
−	6.18	6.32^{cd}	6.91^{abc}	6.94^{abc}
+	6.35	6.37^{bcd}	6.61^{cd}	6.84^{abc}
NC + phytase (PHY; 1,000 U)				
−	6.27	6.29^{d}	6.90^{abc}	6.78^{bc}
+	6.21	6.43^{abcd}	6.63^{cd}	6.67^{c}
NC + protease (PRO; 8,200 U)				
−	6.34	6.28^{d}	7.33^{a}	6.92^{abc}
+	6.28	6.49^{abcd}	6.37^{d}	6.69^{c}
NC + xylanase (XYL; 1,200 U)				
−	6.36	6.32^{cd}	6.25^{d}	6.79^{abc}
+	6.15	6.59^{ab}	6.39^{d}	7.00^{abc}
NC + PHY + PRO				
−	6.24	6.30^{cd}	5.70^{e}	6.92^{abc}
+	6.13	6.55^{abc}	6.73^{bcd}	6.90^{abc}
NC + PHY + XYL				
−	6.18	6.50^{abcd}	6.50^{cd}	7.07^{a}
+	6.25	6.49^{abcd}	6.72^{bcd}	6.75^{bc}
NC + PHY + PRO + XYL				
−	6.12	6.63^{a}	6.73^{bcd}	7.02^{ab}
+	6.12	6.51^{abcd}	7.16^{ab}	6.87^{abc}
Pooled SEM	0.061	0.053	0.096	0.060
Diet				
PC	6.30	6.48	6.74	6.83
NC	6.27	6.35	6.75	6.89
PHY	6.24	6.36	6.77	6.73
PRO	6.31	6.39	6.85	6.81
XYL	6.25	6.45	6.32	6.90
NC + PHY + PRO	6.18	6.42	6.22	6.91
NC + PHY + XYL	6.21	6.50	6.61	6.91
NC + PHY + PRO + XYL	6.12	6.57	6.95	6.94
Pooled SEM	0.043	0.037	0.068	0.042
Vaccination				
Nonvaccinated	6.24	6.38	6.65	6.91
Vaccinated	6.23	6.50	6.65	6.82
Pooled SEM	0.022	0.019	0.034	0.021
P-value				
Diet	0.0519	0.0002	0.0001	0.0106
Vaccination	0.6772	0.0001	0.9272	0.0016
Diet × vaccination	0.0707	0.0017	0.0001	0.0038

[a–e]Means in a column not sharing a common superscript are different ($P \leq 0.05$).

[1]Average number of goblet cells/bird (1 bird/replicate cage) was determined from 4 villi on each of 3 tissue pieces/bird. The bird average was then used to determine mean goblet cell number for each treatment (n = 9).

[2]Nonvaccinated treatment.

[3]Vaccinated treatment.

sure to the initial infection and the resulting intestinal damage may have been slight compared with previously published reports using vaccines in floor pen environments or direct oral challenge of broilers. However, vaccination negatively affected FI and BWG, which is in agreement with other published reports using live coccidia oocyst vaccines (Yi et al., 2005; Parker et al., 2007; Lehman et al., 2009). Vaccination most likely resulted in an inflammatory immune response and the release of interleukin-1 from macrophages (Lillehoj and Trout, 1996), which is known to cause a reduction in FI and feed efficiency (Klasing et al., 1987). Therefore, vaccine-associated reductions in FI and BWG in the current study may have resulted from an inhibition of FI caused by interleukin-1 production, which subsequently reduced BWG without affecting tibia ash.

Significant differences in goblet cell numbers on d 18 related to the interaction of diet and vaccination varied between the duodenum and jejunum. Vaccination significantly increased the number of goblet cells in the duodenum at d 18 when birds were fed xylanase. In addition, nonvaccinated birds fed phytase and xylanase had a larger number of goblet cells in the jejunum at d 18 compared with vaccinated birds fed the same diets. Previous reports including the effect of xylanase on the goblet cell population are inconsistent. Fernandez et al. (2000) reported that xylanase alters mucin composition and carbohydrate expression in the small intestine of

broilers fed wheat-based diets. Wu et al. (2004) reported that xylanase increased the number of goblet cells in the duodenum of healthy broilers fed wheat-based diets. However, supplementing wheat-based diets with the combination of xylanase and β-glucanase (Yaghobfar et al., 2007) or phytase and phytase with xylanase (Wu et al., 2004) decreased goblet cell numbers in the duodenum, jejunum, and ileum of healthy broilers. The innate defense system consists of a mechanical barrier provided by epithelial cells and mucin secreted from goblet cells (Pearson and Brownlee, 2005). Pathologies of coccidiosis include an increase in water and mucus content of the feces and diarrhea (Lillehoj and Trout, 1996). A larger number of goblet cells in the small intestine may be indicative of an increase in mucin secretion or an increase in goblet cell turnover. In the jejunum of 7-d-old broilers, supplemental protease reduced the number of goblet cells in vaccinated birds by approximately 13% compared with nonvaccinated birds. Peek et al. (2009) reported a thicker mucus layer in the small intestine of healthy broilers fed diets supplemented with protease. However, supplemental phytase and protease resulted in 18% fewer goblet cells in nonvaccinated birds as compared with vaccinated ones. Supplemental phytase is known to reduce losses of endogenous mucin associated with phytate (Cowieson et al., 2004). These results would indicate a complex interaction on jejunal goblet cells and possibly mucin secretion and synthesis between dietary phytase and protease in very young broilers.

In conclusion, supplemental phytase and xylanase improved FCR in broilers from d 0 to 18, and phytase improved tibia ash in 7-d-old broilers. Coccidia vaccination reduced FI and BWG, and enzyme supplementation did not alleviate the reduction in growth performance associated with exposure to a mixed *Eimeria* spp. live coccidia oocyst vaccine. The effect of diet and vaccination on goblet cell number was dependent on diet, vaccination treatment, and diet × vaccination interactions as well as on bird age and the intestinal segment.

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