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Enzymes as Feed Additive to Aid in Responses Against *Eimeria* Species in Coccidia-Vaccinated Broilers Fed Corn-Soybean Meal Diets with Different Protein Levels

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ABSTRACT This research aimed to evaluate the effects of adding a combination of exogenous enzymes to starter diets varying in protein content and fed to broilers vaccinated at day of hatch with live oocysts and then challenged with mixed *Eimeria* spp. Five hundred four 1-d-old male Cobb-500 chickens were distributed in 72 cages. The design consisted of 12 treatments. Three anticoccidial control programs [ionophore (IO), coccidian vaccine (COV), and coccidia-vaccine + enzymes (COV + EC)] were evaluated under 3 CP levels (19, 21, and 23%), and 3 unmedicated-uninfected (UU) negative controls were included for each one of the protein levels. All chickens except those in unmedicated-uninfected negative controls were infected at 17 d of age with a mixed oral inoculum of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*. Live performance, lesion scores, oocyst counts, and samples for gut microflora profiles were evaluated 7 d postinfection. Ileal digestibility of amino acids (IDAA) was determined 8 d postinfection. Microbial communities (MC) were analyzed by G + C%, microbial numbers were counted by flow cytometry, and IgA concentrations were measured by ELISA. The lowest CP diets had poorer ($P \leq 0.001$) BW gain and feed conversion ratio in the preinfection period. Coccidia-vaccinated broilers had lower performance than the ones fed ionophore diets during pre- and postchallenge periods. Intestinal lesion scores were affected ($P \leq 0.05$) by anticoccidial control programs, but responses changed according to gut section. Feed additives or vaccination had no effect ($P \geq 0.05$) on IDAA, and diets with 23% CP had the lowest ($P \leq 0.001$) IDAA. Coccidial infection had no effect on MC numbers in the ileum but reduced MC numbers in ceca and suppressed ileal IgA production. The COV + EC treatment modulated MC during mixed coccidiosis infection but did not significantly improve chicken performance. Results indicated that feed enzymes may be used to modulate the gut microflora of cocci-vaccinated broiler chickens.

Key words: broiler, enzyme, crude protein, coccidia vaccination, microbial ecology

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INTRODUCTION

Coccidiosis is still one of the most commonly reported diseases of commercial poultry in spite of advances in chemotherapy, management, nutrition, and genetics (Idris et al., 1997; Lundén et al., 2000; Williams, 2002; McDougald, 2003). Live coccidia vaccines are an alternative to feed medication by enhancing the natural immunity of the chicken via recycling very low doses of coccidial oocysts (Chapman et al., 2002; Williams, 1998, 2002, 2003, 2005). The commercial coccidia vaccines available worldwide contain viable oocysts of at least 3 of the more common *Eimeria* species, such as *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* (Lillehoj and Lillehoj, 2000; Williams, 2002, 2005; Dalloul and Lillehoj, 2005). Under some commercial conditions, live vaccination generally causes a transitory early reduction in growth, which is generally associated with an increased incidence of secondary enteritis and, on occasion, with necrotic enteritis (Chapman et al., 2002; Wages and Kenneth, 2003; Williams, 2005).

The intestinal microflora plays an important role in acquired mucosal immunity and enteritis (Cebra, 1999; Kelly and Conway, 2005). Normal gut microflora in healthy birds inhibits the pathogenicity of *Clostridium perfringens* (Fukata et al., 1991) and modulates the immune responses against coccidia (Lillehoj and Lillehoj, 2000; Dalloul et al., 2003; Dalloul and Lillehoj, 2005). Microbial population profiles in the gastrointestinal tract of broilers are mainly modified by dietary nutrient composition and

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antinutritional factors (Apajalahti et al., 2004) but can also be altered by feed additives (Apajalahti, 2004; Hume et al., 2006; Oviedo-Rondón et al., 2006). Undefined microbial communities (**MC**) and *C. perfringens* (Drew et al., 2004) can be altered when chickens are fed diets with different CP contents that have been supplemented with crystalline amino acids according to the dietary proportions of soybean meal and corn. Diets with variable CP content may also affect the responses of coccidia-vaccinated broilers (Sharma et al., 1973; Richter and Wiesner, 1988).

There are exogenous enzymes commercially available to improve the ileal digestibility, BW gain (**BWG**), and feed conversion of chickens fed corn-soybean meal diets. These products are generally a combination of amylases, xylanases, and proteases (Bedford, 2000a,b; Sohail et al., 2003). Several scientific reports indicate that these exogenous enzymes could additionally decrease the activity of pathogenic bacteria such as *Campylobacter jejuni* and *Salmonella enteritidis* (Bedford, 2000b; Fernandez et al., 2000). Enzymes could act as gut microbial modulators due to the degradation of nonstarch polysaccharides, reduction of the amounts of substrate available for pathogenic bacteria in the ceca, and incremental improvement in the production of natural volatile fatty acids, especially propionic acid (Bedford, 2000b).

We hypothesized that dietary supplementation of exogenous enzymes could improve intestinal gut health and live performance of chickens vaccinated and later infected with live oocysts by enhancing ileal digestibility of amino acids (**IDAA**) or modifying gut microflora and that these responses could be better observed by comparing diets that vary in feedstuff composition.

Two objectives were proposed: 1) to measure the effects of a combination of amylase, protease, and xylanase added over the top in corn-soybean meal diets for broilers vaccinated at day of hatch with live oocysts and later infected with mixed *Eimeria* spp. at 17 d of age and 2) to evaluate the effects of diet protein content on 2 coccidia control strategies, feed medication or vaccination.

MATERIALS AND METHODS

All procedures involving animals were approved by the Stephen F. Austin State University Institutional Animal Care and Use Committee.

Treatments and Broiler Husbandry

A total of 504 one-day-old male Cobb-500 chickens were randomly placed in 72 cages (7 broilers/cage) contained in 3 Petersime battery units (Petersime Incubator Co., Gettysburg, OH). Initial average BW was 42 ± 2 g, and there were no significant ($P \geq 0.05$) differences among treatments. The experimental design consisted of 12 treatments resulting from 3 anticoccidial control programs within each CP level (19, 21, and 23%) plus 3 unmedicated-uninfected (UU) groups, 1 for each CP level that was used as a negative control. The anticoccidial control programs evaluated were diets supplemented with ionophore (IO), coccidia vaccination (COV) at day of hatch, and coccidia vaccination plus dietary supplementation with enzyme combination (COV + EC). Each treatment was randomly assigned to 6 cage replicates.

Three corn-soybean meal basal diets were formulated to obtain 3 levels of CP (Table 1). Dietary Lys, Met, and Thr levels were maintained by addition of crystalline sources to guarantee adequate amino acid concentrations (NRC, 1994). To maintain similar energy levels, additional poultry oil was added, and, consequently, dietary lipid content was changed. Diets were analyzed for CP and total amino acid content, and results are presented in Table 1. Each of these diets was divided into 4 batches. The growth-promotant antibiotic (GPA) bacitracin methylene disalicylate at 50 g/ton (Alpharma Inc., Fort Lee, NJ) and the ionophore monensin Coban-60 at 90 g/ton (Elanco Animal Health, Indianapolis, IN) were added to 1 batch, and the exogenous enzyme Avizyme 1502 feed enzyme system (Danisco Animal Nutrition) was added at 0.05% over the top of the diets in a second lot of feed. The other 2 batches were assigned to treatments fed diets that did not contain any additives. All diets were pelletized, fed as crumbles the first week, and later fed as pellets. Feed and water were provided ad libitum. Room temperature and lighting were controlled to assure bird comfort according to age.

Vaccination and Infection

Half of the chickens (252) were vaccinated at day of hatch by spray in a cabinet with a commercial coccidia vaccine, Advent (Novus International Inc., St. Louis, MO), containing viable attenuated oocysts of *E. acervulina*, *E. maxima*, and *E. tenella*. Brown butcher paper was used as bedding, only in the cages of vaccinated chickens, for the first 3 wk to guarantee oocysts recirculation, but it was changed 3 times during the experiment to guarantee bird comfort. Vaccinated and unvaccinated chickens were assigned to the specific treatments. All broilers except those in UU treatment were infected at 17 d of age with a standard oral inoculum of sporulated oocysts from field isolates (courtesy of David Caldwell, Texas A&M University, College Station) of *E. acervulina*, *E. maxima*, and *E. tenella* at 2×10^5, 1×10^5, and 1×10^5 viable oocysts/mL, respectively.

Data Collection and Analyses

Body weight gain and feed intake (**FI**) were recorded at 17 and 24 d of age. Mortality was recorded twice a day. Feed conversion ratio (**FCR**) was calculated and corrected for BW of mortality. Seven days postinfection, 3 chickens per pen (18/treatment) were humanely killed by cerebrocervical dislocation to observe gut lesion scores (**LS**) and to collect ileal and cecal samples. Lesion scores in the duodenum, midgut, and ceca were evaluated according to Johnson and Reid (1970). Oocyst counts (**OC**) were also performed at 7 d postchallenge from duplicate fecal samples taken from each replicate (12/treatment).

Table 1. Composition (g/kg) and nutrient content of diets with different levels of CP

Ingredients	CP (%)					
	19		21		23	
	%					
Yellow corn grain	60.41		53.49		45.80	
Poultry oil	3.99		5.23		6.61	
Soybean meal (44%)	30.76		36.83		43.48	
Calcium carbonate	1.35		1.35		1.32	
Dicalcium phosphate	1.83		1.76		1.72	
NaCl	0.60		0.60		0.61	
Lys HCl (98%)	0.39		0.21		—	
DL-Met (99%)	0.34		0.29		0.24	
L-Thr (98%)	0.09		—		—	
Vitamin and mineral premix – starter[1]	0.25		0.25		0.25	
Total	100.00		100.00		100.00	
Nutrient content[2] (%)	A[3]	F[3]	A	F	A	F
CP	19.60	18.51	21.40	20.05	23.40	22.97
TSAA	0.92	0.96	0.92	0.96	0.92	1.10
Met	0.61	0.57	0.59	0.55	0.56	0.58
Lys	1.27	1.30	1.27	1.28	1.27	1.34
Thr	0.82	0.73	0.82	0.73	0.90	0.85
Trp	0.28	0.19	0.32	0.25	0.36	0.26
Val	0.89	0.83	0.99	0.94	1.09	1.05
Leu	1.65	1.56	1.78	1.70	1.93	1.90
Arg	1.28	1.16	1.44	1.32	1.60	1.54
Ca	1.00	1.00	1.00	1.00	1.00	1.00
Available P	0.45	0.45	0.45	0.45	0.45	0.45

[1]Poultry Science Mineral and Vitamin Premix (Animal Science Products, Nacogdoches, TX) provided the following per kilogram of diet: vitamin A (from vitamin A acetate), 7,714 IU; cholecalciferol, 2,204 IU; vitamin E (from DL-α tocopheryl acetate), 16.53 IU; vitamin B_{12}, 0.013 mg; riboflavin, 6.6 mg; niacin, 39 mg; pantothenic acid, 10 mg; choline, 465 mg; menadione (from menadione dimethylpyrimidinol), 1.5 mg; folic acid, 0.9 mg; thiamin (from thiamine mononitrate), 1.54 mg; pyridoxine (from pyridoxine hydrochloride), 2.76 mg; D-biotin, 0.066 mg; ethoxyquin, 125 mg; and Se, 0.1 mg. The following minerals were provided per kilogram of diet: Mn (from $MnSO_4 \cdot H_2O$), 100 mg; Zn (from $ZnSO_4 \cdot 7\ H_2O$), 100 mg; Fe (from $FeSO_4 \cdot 7\ H_2O$), 50 mg; Cu (from $CuSO_4 \cdot 5\ H_2O$), 10 mg; and I [from Ca $(IO_3)_2 \cdot H_2O$], 1 mg.

[2]All diets were formulated to contain 3,108 kcal of ME/kg.

[3]A = analyzed nutrient contents; F = formulated nutrient contents.

Fecal samples were homogenized and diluted in a saturated NaCl solution at a ratio of 1:10 before counting in 2 McMaster chambers per sample to determine the number of oocysts per gram of feces (Hodgson, 1970).

Samples of ileal and cecal contents were collected into 50-mL conical tubes within 10 min after chickens were euthanized, frozen in liquid N, and kept at –70°C until analyses were performed.

Protein and Amino Acid Digestibility Study

A digestibility study was conducted at the end of the experiment 8 d postchallenge. Experimental diets contained 0.8% Celite (Celite Corp., Lompoc, CA) as an internal marker. Four birds per cage (24/treatment) were euthanized, and distal ileal samples were collected. Samples were quickly frozen in liquid N and kept at –70°C until lyophilization. After all ileal digesta samples were completely lyophilized, 2 replications were pooled to obtain sufficient digesta to complete all necessary analyses, then a final 3 replicates per treatment were analyzed. Diets and lyophilized ileal digesta samples were analyzed for insoluble ash, CP, and total amino acid contents. All values were expressed on a DM basis. Nutrient digestibility was calculated from the following formula:

$$\text{Digestibility \%} = 100 - [100 \times (\%\text{Marker diet}/\%\text{Marker digesta}) \times (\%\text{Nutrient digesta}/\%\text{Nutrient diet})]$$

IgA, MC Profiling by G + C%

Frozen ileal and cecal samples were sent to Danisco Innovation Laboratory. Immunoglobulin A concentrations were measured by ELISA as in Kettunen et al. (2003). The total number of bacterial cells was measured by flow cytometry by a method described in Apajalahti et al. (2002). The G + C% profiles of the MC were analyzed according to Apajalahti et al. (2001).

Statistical Analysis

Pens served as experimental units for all variables. Means for each pen were computed during the pre- and postinfection periods and analyzed separately according to the following statistical models:

Preinfection period:

$$Y_{ijkl} = CP_i + V_j + CP_i \times V_j + A_{k(j)} + CP_i \times A_{k(j)} + e_{ijkl}$$

Postinfection period:

$$Y_{ijklm} = Inf_i + CP_{j(i)} + V_{k(i)} + A_{l(k \times i)} + CP_j \times V_{k(i)} + CP_j \times A_{l(k \times i)} + e_{ijklm}$$

where Y = the observations of response variables; CP = the 3 CP levels; V = the effect of cocci vaccination or not; A = the effect of the 2 groups of additives used (IO + GPA or EC); Inf = the effect of mixed coccidia infection at 17 d of age; and e = the experimental error associated to each observation.

Percentage mortality data were transformed by the arcsine method before analysis, and final data are presented as natural numbers. All tests were carried out using level of significance at $\alpha = 0.05$. The GLM procedure of the SAS system (SAS Institute, 2001) was used to produce statistics necessary for ANOVA. Generally, this ANOVA involved partitioning total sum of squares into orthogonal components reflecting variability due to the sources specified in the pre- and postinfection models described above. This was accomplished either by nesting effects within the MODEL statement or by specifying appropriate contrast matrices using the CONTRAST statement. In multiple comparisons among treatment means, Tukey's test was used to control the experiment-wise error rate at $\alpha = 0.05$. Because the oocyst yields were not normally distributed, they were transformed using ln(x + 1) before analyses, and data were presented as natural numbers. Lesion scores were analyzed within each 1 of the 3 gut sections and as an average of total LS using a χ^2 by the Kruskal–Wallis test method (Sall and Lehman, 1996). Because no OC or LS was observed in the UU treatments, these data were analyzed only for infected treatments using the statistical model that does not include infection.

RESULTS

Live Performance

Preinfection Period. During the preinfection period (Table 2), significant independent effects of CP levels ($P \leq 0.001$) and vaccination ($P \leq 0.001$) were observed for BWG. The growth rate of chickens fed the 19% CP diets was significantly lower than those fed the 21 and 23% CP diets. The addition of IO + GPA did not improve ($P \geq 0.05$) growth or FCR compared with the UU negative control treatments. Vaccination with live *Eimeria* spp. oocysts at day of hatch affected FI ($P \leq 0.001$) and caused a reduction in BWG ($P \leq 0.001$) and FCR ($P \leq 0.05$) independently of dietary CP level. This reduction in the performance of coccidia-vaccinated broilers was not improved ($P \geq 0.05$) by dietary supplementation of this enzyme complex. Enzyme supplementation of coccidia-vaccinated chickens fed 23% CP diets caused lower FI ($P \leq 0.05$). Feed conversion rate improved ($P \leq 0.001$) as the CP level increased.

Postinfection Period. The average FI and BWG of all treatments infected with mixed *Eimeria* spp. were reduced ($P \leq 0.001$) by 21 and 45%, respectively, whereas the FCR increased by 43% compared with the average of UU controls (Table 3). However, no significant ($P \geq 0.05$) effects on mortality due to treatments were observed (data not shown). The *Eimeria* spp. infection increased the CV of all response variables measured. Body weight gain and FCR of UU chickens during this postinfection period were similar ($P \geq 0.05$) across all dietary CP levels. Nevertheless, FI of these UU broilers was lower when fed 23% CP diets. Body weight gain, FI, and FCR were affected ($P \leq 0.05$) by the interaction between CP level and vaccination within infection. In general, the average FI of infected treatments decreased ($P \leq 0.05$) as the dietary CP content increased. However, although the FI of all vaccinated treatments improved as the CP level increased, the FI of chickens fed diets supplemented with IO + GPA had the opposite trend. Chickens fed diets containing 23% CP had the lowest FI among the infected chickens fed IO + GPA diets. Diets with 23% CP also supported the best FI and FCR among all vaccinated chickens after coccidia infection. The live performance of IO + GPA broilers was better than the performance of all coccidia-vaccinated broilers during this postchallenge period but not similar ($P \geq 0.05$) to the UU controls. Enzyme supplementation did not improve ($P \geq 0.05$) live performance 7 d after mixed *Eimeria* infection.

OC and LS

The oocyst shedding counts decreased ($P \leq 0.05$) as the dietary CP level increased (Table 4). No other significant effects of treatments were observed on OC.

Lesion scores were recorded for 3 separate sections of the intestine (Figure 1), and the average of these LS is presented in Table 4. An evaluation of individual LS in the 3 gut sections showed no significant effects of CP, COV ($P \geq 0.05$), or CP × COV were observed, but enzyme supplementation reduced ($P \leq 0.001$) average LS in vaccinated broilers. Additionally, other effects of additives were observed in the midgut and ceca (Figure 1). No significant differences due to treatments were observed in the duodenal section. In the jejunum and ileum sections, chickens fed diets supplemented with IO had significantly lower LS ($P \leq 0.05$) than the ones observed in chickens from vaccinated treatments. The addition of enzyme did not ($P \geq 0.05$) reduce LS in the midintestine. Nevertheless, coccidia-vaccinated chickens fed diets supplemented with the enzyme complex (COV + EC) had lower ($P \leq 0.01$) LS in the ceca than COV chickens, without being different ($P \geq 0.05$) from the LS observed in chickens fed IO + GPA diets (Figure 1).

IDAA

The average IDAA of all chickens infected with mixed coccidia was reduced ($P \leq 0.001$) in 7.9% (85.6 vs. 77.7%) 8 d after infection (Table 5). The mean ileal digestibility of Lys, Met, Ile, Val, and Leu decreased ($P \leq 0.05$) in all infected broilers as the dietary CP level increased. In general, vaccination or feed additives did not improve ($P \geq 0.05$) the IDAA (Table 5). Nevertheless, vaccinated

Table 2. Effects of dietary CP level, vaccination, and feed additives on BW gain, feed intake, and feed conversion during the precoccidia infection period (1 to 17 d)[1]

	BW gain (g/d)				Feed intake (g/d)				Feed conversion ratio (g:g)			
CP (%)	19	21	23	Mean	19	21	23	Mean	19	21	23	Mean
Unvaccinated												
UU[2]	31.3	33.5	34.5	33.1	44.6	44.8	43.4	44.3	1.427	1.337	1.257	1.341
IO + GPA[3]	30.2	32.6	33.8	32.2	43.1	44.5	42.6	42.9	1.425	1.319	1.261	1.335
Mean unvaccinated	30.8	33.1	34.2	32.7^{A}	43.9	44.7	42.9	43.8^{A}	1.426	1.329	1.260	1.338
Vaccinated												
COV[4]	28.1	29.9	31.9	29.9	40.5^{ab}	41.3^{ab}	43.4^{a}	41.7	1.436	1.388	1.363	1.396
COV + EC[5]	28.5	31.8	30.8	30.1	42.6^{a}	42.3^{a}	39.2^{b}	41.4	1.496	1.337	1.277	1.370
Mean vaccinated	28.3	30.8	31.3	30.0^{B}	41.5	41.8	41.3	41.5^{B}	1.466	1.363	1.321	1.383
Mean	29.5^{b}	31.9^{a}	32.7^{a}	31.3	42.7	43.2	42.1	42.7	1.453^{a}	1.348^{bc}	1.301^{c}	1.367
SEM		1.1				0.7				0.027		
CV (%)		6.94				6.44				3.51		
Source of variation	df	*P*-value										
CP	2		<0.0001				0.4019				<0.0001	
COV	1		<0.0001				0.0008				0.0509	
CP × COV	2		0.9295				0.7441				0.9765	
ADD[6] (COV)	2		0.4874				0.5910				0.5623	
IO + GPA	1[7]			0.2552				0.3442				0.2858
EC (COV)	1			0.7147				0.6981				0.9594
CP × ADD (COV)	4		0.6154				0.0663				0.3941	
CP × IO + GPA	2			0.9829				0.8678				0.9613
CP × EC (COV)	2			0.2741				0.0149				0.1389

[a–c]Means within a row lacking a common lowercase superscript are significantly different ($P < 0.05$).

[A,B]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).

[1]Means represent 6 replicates per treatment.

[2]UU = unmedicated-uninfected broilers.

[3]IO + GPA= ionophore monensin (Coban-60 at 90 g/ton, Elanco Animal Health, Indianapolis, IN) and antibiotic bacitracin methylene disalicylate at 50 g/ton (Alpharma Inc., Fort Lee, NJ).

[4]COV = cocci-vaccinated with Advent (Novus International Inc., St. Louis, MO) at 1 d of age by spray-fed diets without feed additives.

[5]EC = enzyme combination designed for corn-soybean meal diets, Avizyme 1502 (Danisco Animal Nutrition).

[6]ADD = feed additives; included enzyme (COV + EC) and ionophore plus the growth-promotant antibiotic (IO + GPA).

[7]Indentation implies that sums of squares above are being partitioned into orthogonal components.

broilers (COV and COV + EC) had better ileal digestibility of Arg than chickens fed IO + GPA diets (87.1 ± 0.8% vs. 84.1 ± 1.2%).

IgA and Gut MC

The concentration of IgA showed high variation between the replicate pools of the treatments. In the ileum, the mixed coccidia infection suppressed ($P \leq 0.001$) IgA production, whereas in ceca, IgA was affected by the interaction between CP with vaccine or with feed additives only in infected chickens (Table 6). The highest cecal IgA concentrations among IO + GPA chickens were observed in the ones fed 23% CP.

Microbial numbers in the ileum were reduced in the infected treatments; the response varied due to the CP and additive interaction mainly in the coccidia-vaccinated treatments (COV and COV + EC). Ileal MC numbers of COV + EC chickens were reduced when fed 23% CP diets. Microbial cell numbers were reduced ($P \leq 0.001$) in the ceca after the challenge (Table 6). Cecal MC numbers were higher ($P \leq 0.05$) in coccidia-vaccinated chickens than in IO + GPA chickens.

Microbial profiles described by G + C% were affected ($P \leq 0.05$) by both dietary CP level and vaccination or feed additives (Figure 2). On average, treatments infected with mixed coccidia species hosted gut MC characterized by higher relative abundance of bacteria in the 50 to 80 G + C% range when the diet had either 21 or 23% CP ($P \leq 0.01$). Cocci-vaccinated chicks supplemented with enzymes (COV + EC) fed diets 19% CP showed very similar G + C% profiles related to the UU controls (Figure 2, panel A). The *t*-test comparing COV and COV + EC treatments indicated that microbial profiles changed ($P \leq 0.001$) due to enzyme supplementation in all G + C% increments except in those from 60 to 69% in chickens fed 23% CP diets (Figure 2, panel C).

DISCUSSION

The transient reduction of live performance frequently observed in broilers vaccinated with live *Eimeria* oocysts at day of hatch was not significantly ameliorated by dietary supplementation with this enzyme combination under the conditions of the present experiment. Feed additives IO + GPA did not improve the performance of unvaccinated broilers. The dietary enzyme supplementation did not significantly improve the live performance of coccidia-vaccinated chickens.

Dietary enzyme supplementation of coccidia-vaccinated broilers did not help to considerably reduce the oocyst shedding but supported a gut environment that

Table 3. Effects of dietary CP level, coccidia vaccination, feed additives, and mixed *Eimeria* spp. infection at 17 d of age on average daily BW gain, feed intake, and feed conversion after mixed coccidia challenge (17 to 24 d)[1]

		BW gain (g/d)				Feed intake (g/d)				Feed conversion ratio (g:g)			
CP (%)		19	21	23	Mean	19	21	23	Mean	19	21	23	Mean
UU[2]		50.0[a]	50.6[a]	51.0[a]	50.6[A,X]	89.0[a]	87.7[ab]	86.4[b]	87.7[A,X]	1.787[c]	1.752[c]	1.713[c]	1.751[B,Z]
Infected at 17 d													
IO + GPA[3]		33.0[b]	31.6[bc]	26.7[cde]	30.3[Y]	78.3[c]	74.7[cd]	66.3[g]	73.1[Y]	2.233[b]	2.429[ab]	2.517[ab]	2.393[Y]
COV[4]		24.1	23.0	29.4	25.6	68.4	64.3	66.9	66.6	2.743	2.804	2.322	2.617
COV + EC[5]		25.6	27.7	28.4	27.1	65.3	69.9	68.0	67.7	2.614	2.583	2.437	2.545
Mean vaccinated		24.8[e]	25.3[de]	28.9[bcd]	26.4[Z]	66.8[f]	67.1[e]	67.4[d]	67.1[Z]	2.679[a]	2.694[a]	2.379[b]	2.584[X]
Mean infected		27.6	27.4	28.2	27.7[B]	70.6[x]	69.6[xy]	67.1[y]	69.1[B]	2.530	2.605	2.425	2.518[A]
Mean		33.2	33.2	33.9	33.4	75.3	74.2	71.9	73.8	2.344	2.392	2.247	2.327
SEM			0.8				1.1				0.148		
CV (%)			14.78				7.95				14.03		
Source of variation	df	*P*-value											
INF[6]	1		<0.0001				<0.0001				<0.0001		
CP (INF)	4		0.9958				0.1699				0.7657		
UU	2[7]				0.9387				0.7376				0.9262
INF	2				0.8267				0.0246				0.5525
COV (INF)	1		0.0065				0.0008				0.0486		
ADD[8] (COV × INF)	1		0.3099				0.5381				0.4776		
CP × COV (INF)	2		0.0121				0.0117				0.0430		
CP × ADD [COV × INF]	2		0.3774				0.1928				0.4315		

[a–g]Means across columns and rows lacking a common lowercase superscript are significantly different ($P < 0.05$).

[x,y]Means within a row lacking a common lowercase superscript are significantly different ($P < 0.05$).

[A,B]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$) to compare UU and infected treatments.

[X–Z]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).

[1]Means represent 6 replicates per treatment.

[2]UU = unmedicated-uninfected broilers.

[3]IO + GPA = ionophore monensin (Coban-60 at 90 g/ton, Elanco Animal Health, Indianapolis, IN) and growth-promotant antibiotic bacitracin methylene disalicylate at 50 g/ton (Alpharma Inc., Fort Lee, NJ).

[4]COV = cocci-vaccinated with Advent (Novus International Inc., St. Louis, MO) at 1 d of age by spray-fed diets without feed additives.

[5]EC = enzyme combination designed for corn-soybean meal diets, Avizyme 1502 (Danisco Animal Nutrition).

[6]INF = effect of infection with a mixed solution of approximately 2×10^5 oocysts of *Eimeria acervulina*, 1×10^5 oocysts of *Eimeria maxima*, and 1×10^5 oocysts of *Eimeria tenella* per bird at 17 d of age.

[7]Indentation implies that sums of squares above are being partitioned into orthogonal components.

[8]ADD = feed additives; included enzyme (COV + EC) and ionophore plus the growth-promotant antibiotic (IO + GPA).

reduced the average intestinal LS observed 8 d postinfection in all 3 intestinal sections and especially in the cecal section. Bedford (2000b) had described similar responses in reduction of lesions caused by *E. acervulina* in chickens fed diets supplemented with enzymes, and these properties have been attributed to higher production of volatile fatty acids that reduce pathogenicity of *E. tenella* or *Salmonella* spp. colonization (Apajalahti, 2004). The mixed *Eimeria* spp. infection evaluated in the present experiment caused more severe and complex changes in the intestinal ecosystem than what can be observed during an infection with a single species of *Eimeria*. The effects of multiple *Eimeria* infections cannot be easily explained due to the damage in the mucosa that individual *Eimeria* spp. have according to their tropism and the consequent effects on the systemic immune response, gastrointestinal physiology, and nutrient substrates available to the subsequent sections of the digestive tract. However, taking into consideration that all vaccines commercially available contain oocysts of at least 3 *Eimeria* species, and field challenges are caused for more than 1 *Eimeria* species (Chapman et al., 2002; Williams, 2002, 2005), it is important to evaluate the complex effects of mixed infection.

Neither the vaccinated chickens nor the IO + GPA chickens had live performance or digestibility similar to the UU controls after the mixed infection. Data indicated that the enzymes had no significant direct effect on ileal CP or amino acid digestibility of coccidia-vaccinated broilers; however, carbohydrate, lipid, and protein substrates available to microbiota communities in the ceca seems to be altered, resulting in MC similar to the ones observed in the UU controls (Bedford, 2000b; Apajalahti, 2004). The lack of response in IDAA observed in this experiment could be due to mucosa damage, increased passage rate that affects the amino acid absorption, or increased sloughing of mucosa and blood cells during this period (Apajalahti, 2004) that confounded the real amounts of amino acid present in the digesta collected in the distal ileum and increased sample variability (Persia et al., 2006).

The present study confirms the findings of Kettunen and Rautonen (2005) that *E. maxima* infection suppressed the concentration of IgA in the ileum. On the other hand, the present experiment suggested that cecal *E. tenella* infection, even if manifested by high LS, does not have to decrease the cecal production of Ig. Dietary CP levels,

Table 4. Effects of dietary CP level, vaccination, and feed additives on oocyst counts and average lesions scores evaluated 7 d post mixed *Eimeria* spp. infection (24 d of age)

	Oocyst count[1] (10^3/g of excreta)				Average lesion scores[2]			
CP (%)	19	21	23	Mean	19	21	23	Mean
UU[3]	0	0	0	0	0	0	0	0
Infected								
IO + GPA[4]	6.73	4.58	2.58	4.63	1.33	1.25	1.29	1.29
COV[5]	3.59	4.00	5.67	4.42	1.79	1.71	2.00	1.83^{X}
COV + EC[6]	8.79	3.70	1.42	4.64	1.33	1.54	1.08	1.32^{Y}
Mean COV	6.19	3.85	3.54	4.53	1.56	1.63	1.54	1.58
Mean	6.47^{a}	4.22^{ab}	3.06^{b}	4.56	1.49	1.50	1.46	1.48
SEM		2.42				0.17		

Source of variation	df	*P*-value				
CP	2	0.0152			0.9721	
COV	1	0.9996			0.0559	
CP × COV	2	0.7845			0.7289	
ADD[7] (COV)	2	0.9443			0.0007	
IO + GPA	1[8]		0.8403			0.9298
EC (COV)	1		0.8603			<0.0001
CP × ADD (COV)	4	0.2872			0.1442	
CP × IO + GPA	2		0.9143			0.4017
CP × EC (COV)	2		0.1652			0.4541

[a,b]Means within rows lacking common lowercase superscripts are significantly different ($P < 0.05$).

[X,Y]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).

[1]Total oocyst counts per gram of excreta. Means represent 6 replicates per treatment (2 samples/cage).

[2]Kruskal-Wallis test was used to evaluate lesion score data from each gut section ($P < 0.05$). Means represent an average of all three intestinal segments of 3 birds/cage averaged to obtain 6 replicates per treatment.

[3]UU = unmedicated-uninfected broilers. All other treatments were orally infected with a mixed solution of approximately 2×10^5 oocysts of *Eimeria acervulina*, 1×10^5 oocysts of *Eimeria maxima*, and 1×10^5 oocysts of *Eimeria tenella* per bird at 17 d of age.

[4]IO + GPA = ionophore monensin (Coban-60 at 90 g/ton, Elanco Animal Health, Indianapolis, IN) and growth-promotant antibiotic bacitracin methylene disalicylate at 50 g/ton (Alpharma Inc., Fort Lee, NJ).

[5]COV = coccidia vaccinated with Advent (Novus International Inc., St. Louis, MO) at 1 d of age by spray-fed diets without feed additives.

[6]EC = enzyme combination designed for corn-soybean meal diets, Avizyme 1502 (Danisco Animal Nutrition).

[7]ADD = feed additives; included enzyme (COV + EC) and ionophore plus the growth-promotant antibiotic (IO + GPA).

[8]Indentation implies that sums of squares above are being partitioned into orthogonal components.

vaccination, and the feed additives affected the cecal intestinal IgA but had no effect on ileal IgA concentrations in coccidia-vaccinated broilers. However, dietary CP levels, vaccination, and feed additives had significant interacting effects on the microbial numbers in the ileum and the MC profiles of coccidia-vaccinated broilers (Table 6, Figure 2). Enzyme supplementation helped to modulate the cecal microflora, especially in chickens fed diets with 19 and 21% CP toward the values of UU control chickens. Although no significant ($P \geq 0.05$) beneficial effects of enzyme addition were observed in live performance, enzyme supplementation caused an important numerical improvement in FCR between the COV and COV + EC treatments (2.804 vs. 2.583) in coccidia-vaccinated chickens fed 21% CP diets. Chickens fed IO + GPA diets with 19% CP had the highest daily BWG (33 g/d) after infection, and all chickens fed these low-protein diets suffered no significant shifts in cecal MC compared with the UU controls in spite of the mixed *Eimeria* spp. infection. These results give more evidence about the importance of cecal microflora modulation. Our previous research (Hume et al., 2006; Oviedo-Rondón et al., 2006) related to intestinal ecology dynamics of broilers after mixed coccidia challenges indicated that modulation of cecal microflora is somewhat related to adequate FI needed to maintain growth under this immunological stress.

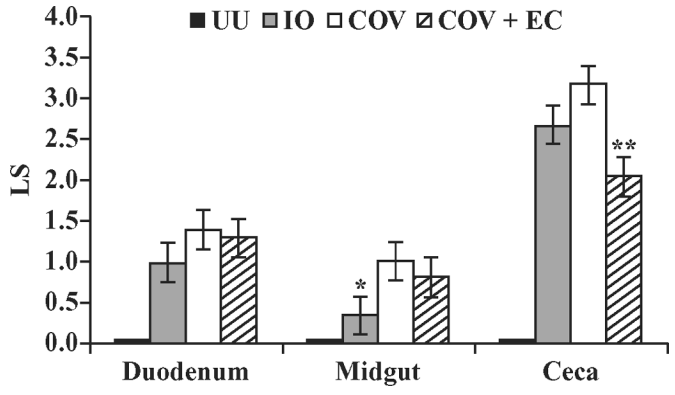

Figure 1. Intestinal lesion scores (LS) observed in broilers 7 d after mixed *Eimeria* spp. challenge. Anticoccidial control programs evaluated were compared independently of CP content of the diets. UU = unmedicated-uninfected controls; IO = ionophore monensin, COV = coccidia vaccination; and COV + EC = coccidia vaccination + dietary enzyme supplementation. *$P \leq 0.05$ and **$P \leq 0.01$.

Table 5. Effect of infection, vaccination, and feed additives on broiler ileal CP and amino acid digestibilities (%) 8 d after challenge (25 d of age) with mixed coccidian oocysts

		Infected				
			Vaccinated			
Nutrient	UU[2]	IO + GPA[3]	Without feed	Enzyme	*P*-value[1]	CP (%)
CP	83.1[a] ± 0.5	73.9[b] ± 1.3	75.4[b] ± 1.2	76.0[b] ± 0.8	0.0001	3.20
Lys	90.0[a] ± 0.5	80.9[b] ± 1.7	82.7[b] ± 1.4	82.9[b] ± 1.0	0.0001	3.11
Met	94.1[a] ± 0.2	87.3[b] ± 1.2	88.7[b] ± 0.8	88.4[b] ± 0.7	0.0001	2.49
Cystine	81.1[a] ± 0.8	70.8[b] ± 1.5	72.9[b] ± 1.5	73.4[b] ± 1.1	0.0001	4.09
Thr	78.6[a] ± 0.8	69.5[b] ± 1.9	70.4[b] ± 1.7	70.5[b] ± 0.9	0.0001	4.62
Trp	84.1[a] ± 0.7	79.7[b] ± 1.1	81.4[b] ± 1.0	79.5[b] ± 1.7	0.0225	3.81
Ile	85.0[a] ± 0.5	74.8[b] ± 1.4	76.2[b] ± 1.4	77.5[b] ± 0.8	0.0001	3.45
Val	83.2[a] ± 0.5	71.7[b] ± 1.6	73.7[b] ± 1.4	74.2[b] ± 0.8	0.0001	3.59
Leu	86.2[a] ± 0.4	77.4[b] ± 1.4	78.9[b] ± 1.3	79.4[b] ± 0.8	0.0001	3.20
Arg	91.5[a] ± 0.4	84.1[b] ± 1.2	87.3[b] ± 0.9	86.9[b] ± 0.8	0.0001	3.11
Gly	80.2[a] ± 0.6	69.0[b] ± 1.5	70.6[b] ± 1.5	71.6[b] ± 0.9	0.0001	4.00
Ser	83.3[a] ± 1.0	74.1[b] ± 1.5	76.6[b] ± 1.1	76.2[b] ± 0.9	0.0001	3.97
Mean amino acids[4]	85.6[a] ± 2.0	76.5[b] ± 2.2	78.3[b] ± 2.3	78.3[b] ± 2.2		
CV (%)	4.62	6.47	6.58	6.32		

[a,b]Means ± SE within a row lacking a common lowercase superscript differ ($P < 0.05$).

[1]Effect of infection on ileal digestibility of amino acids.

[2]UU = unmedicated-uninfected broilers.

[3]IO + GPA = ionophore monensin (Coban-60 at 90 g/ton, Elanco Animal Health, Indianapolis, IN) and growth-promotant antibiotic bacitracin methylene disalicylate at 50 g/ton (Alpharma Inc., Fort Lee, NJ).

[4]Average of 18 amino acids.

Dietary protein level had a significant effect on BWG and FCR during the preinfection period. These diets were formulated to fulfill requirements of essential AA with synthetic AA supplementation. However, the analyzed CP and AA values indicated that 19 and 21% CP diets had lower amino acid contents than the formulated values (Table 1) except for Lys and TSAA. When the CP level of broiler starter diets is reduced below 19% CP, it is common to observe significant reductions in live performance, although Lys and Met requirements are fulfilled (Si et al., 2004; Jiang et al., 2005).

Diets with the lowest CP content tested in this experiment failed to support broiler live performance similar to the one observed in chickens fed diets with 21 or 23% CP during the preinfection period. However, it is important to notice that during the 7 d postinfection, UU controls had similar BWG and FCR in spite of the CP content and even lower FI with the 23% CP. The BWG and FI of chickens fed IO + GPA diets significantly deteriorated as the CP level of the diet increased. In contrast, chickens cocci-vaccinated had the best BWG and numerically better FCR when they were fed diets with the highest CP level (23% CP). It has been reported that high dietary CP content increases broiler gut *C. perfringens* populations (Drew et al., 2004), necrotic enteritis incidence (Williams, 2005), mortality in *E. tenella*-infected chickens, and *E. acervulina* oocyst shedding (Sharma et al., 1973) in nonvaccinated chickens. However, the data presented here showed that chickens fed low-protein diets had the highest total oocyst shedding counts 7 d after mixed *Eimeria*

Table 6. Effect of dietary CP content of basal diets on broiler ileal CP and amino acid digestibilities (%) 8 d after an infection (25 d of age) with mixed coccidian oocysts

	Dietary CP (%)				
Nutrient	19	21	23	*P*-value[1]	CV (%)
CP	77.7 ± 1.1	77.3 ± 1.2	76.2 ± 1.6	NS	3.20
Lys	86.7[a] ± 0.9	84.4[a] ± 1.2	81.2[b] ± 1.8	0.0002	3.20
Met	91.2[a] ± 0.7	89.7[a] ± 0.9	87.9[b] ± 1.2	0.0014	2.52
Cystine	74.0 ± 1.4	73.7 ± 1.6	76.0 ± 1.7	NS	4.14
Thr	73.2 ± 1.3	72.0 ± 1.3	71.5 ± 2.1	NS	4.63
Trp	80.5[a] ± 1.1	83.0[a] ± 0.8	80.1[b] ± 1.3	NS	3.82
Ile	79.6 ± 1.1	78.8 ± 1.3	76.8 ± 1.9	NS	3.53
Val	76.2 ± 1.3	76.9 ± 1.4	74.0 ± 2.1	NS	3.62
Leu	81.9[a] ± 0.9	80.8[ab] ± 1.2	78.8[b] ± 1.7	0.0524	3.21
Arg	87.5 ± 0.9	86.7 ± 1.2	88.0 ± 1.0	NS	3.11
Asp	79.1[a] ± 1.0	78.2[ab] ± 1.4	77.5[b] ± 1.7	0.0524	3.53
Mean amino acids[2]	80.4[a] ± 2.2	80.0[a] ± 2.2	78.9[b] ± 2.1		
CV (%)	6.26	5.94	5.77		

[a,b]Means ± SE within a row lacking a common lowercase superscript differ ($P < 0.05$).

[1]Effect of CP on ileal digestibility of amino acids of infected treatments.

[2]Average of 18 amino acids.

Table 7. Effects of infection, dietary CP level, coccidia vaccination, and feed additives on IgA concentration and microbial cell counts of the ileal and cecal digesta of broilers 7 d after a mixed *Eimeria* spp. infection (24 d of age)

		IgA (μg/g of digesta)								Microbial cells							
		Ileum				Ceca				Ileum (10^9 cells/g)				Ceca (10^{11} cells/g)			
CP (%)		19	21	23	Mean	19	21	23	Mean	19	21	23	Mean	19	21	23	Mean
UU[1]		738	980	862	860^{A,X}	3,115^{b}	4,028^{b}	3,722^{b}	3,621	2.09^{abc}	1.29^{bc}	1.33^{bc}	1.57	2.71	2.26	2.39	2.45^{A,X}
Infected																	
IO + GPA[2]		444	496	443	461	2,655^{b}	4,507^{b}	6,547^{a}	4,570	1.78^{bc}	1.24^{bc}	1.57^{bc}	1.53	0.87	1.19	0.78	0.95^{Z}
COV[3]		433	454	338	417	3,912^{b}	8,336^{a}	4,158^{b}	5,486	1.36^{bc}	2.01^{abc}	2.09^{abc}	1.82	1.27	1.28	1.50	1.35
COV + EC[4]		389	506	288	394	5,646^{ab}	3,761^{b}	2,571^{b}	3,993	2.62^{a}	2.39^{ab}	1.09^{c}	2.03	2.03	1.59	1.48	1.70
Mean COV		411	480	313	401	4,779^{ab}	6,073^{a}	3,365^{b}	4,739	1.99	2.20	1.59	1.92	1.65	1.43	1.49	1.52^{Y}
Mean INF[5]		427	488	377	431^{B}	3,717	5,290	4,956	4,654	1.88	1.72	1.58	1.72	1.26	1.31	1.13	1.23^{B}
Mean		501	609	483	531	3,832	5,170	4,249	4,417	1.92	1.88	1.58	1.79	1.39	1.35	1.25	1.33^{B}
Pooled SEM			119				1,042				0.56				0.20		
CV (%)			15.5				14.4				15.8				14.7		
Source of variation	df	*P*-value															
INF	1		0.0002				0.1603				0.5019				0.0002		
CP (INF)	4		0.5102				0.5028				0.5057				0.8635		
UU	2[6]			0.5791				0.8224				0.3149				0.6392	
INF	2			0.3876				0.2262				0.6810				0.8253	
COV (INF)	1		0.4935				0.8220				0.1793				0.0336		
ADD[7] (COV × INF)	1		0.8900				0.1048				0.5313				0.2319		
CP × COV (INF)	2		0.8400				0.0234				0.3796				0.6253		
CP × ADD (COV × INF)	2		0.8930				0.0318				0.0429				0.5305		

[a–c]Means across columns and rows lacking a common lowercase superscript are significantly different ($P < 0.05$).

[A,B]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).

[X–Z]Means across rows lacking a common uppercase superscript are significantly different ($P < 0.05$).

[1]UU= unmedicated-uninfected broilers.

[2]IO + GPA = ionophore monensin (Coban-60 at 90 g/ton, Elanco Animal Health, Indianapolis, IN) and growth-promotant antibiotic bacitracin methylene disalicylate at 50 g/ton (Alpharma Inc., Fort Lee, NJ).

[3]COV = cocci-vaccinated with Advent (Novus International Inc., St. Louis, MO) at 1 d of age by spray-fed diets without feed additives.

[4]EC = enzyme combination designed for corn-soybean meal diets, Avizyme 1502 (Danisco Animal Nutrition).

[5]INF = effect of infection with a mixed solution of approximately 2×10^5 oocysts of *Eimeria acervulina*, 1×10^5 oocysts of *Eimeria maxima*, and 1×10^5 oocysts of *Eimeria tenella* per bird at 17 d of age.

[6]Indentation implies that sums of squares above are being partitioned into orthogonal components.

[7]ADD = feed additives; included enzyme (EC) and ionophore plus the growth-promotant antibiotic (IO + GPA).

spp. infection. The data presented here were obtained in a battery trial, and gut MC may differ from typical commercial conditions with higher challenges of *Clostridium* spp. Results of the present experiment clearly indicated that dietary composition can alter gut MC and the live performance of chickens vaccinated against coccidia or supplemented with feed additives used to control coccidiosis. The initial low-CP diet may compromise immunological mechanisms that reduce the resistance of coccidia-vaccinated broilers to infection and increase oocyst shedding.

Digestibility data (Table 7) indicated that chickens fed 23% CP diets had ($P \leq 0.05$), on average, 1.5% more undigested protein and amino acids in the ceca than chickens fed other types of diets independently of feed additive used. These undigested nutrients in the ceca seem to be more prejudicial for the performance of chickens fed IO + GPA diets than for COV or COV + EC chickens 7 d after infection (Table 3). These results related to diet composition and nutrient substrate changes during 2 periods of life, pre- and postinfection with mixed coccidia, may explain the variation in results observed under field or experimental conditions when using different anticoccidial strategies, as it was discussed by Sharma et al. (1973), Drew et al. (2004), or Williams (2005).

In conclusion, results indicated that dietary supplementation with the exogenous enzyme combination did not significantly improve the performance of coccidia-vaccinated broilers during pre- and postinfection periods. The beneficial effects observed to reduce LS, especially against *E. tenella*, may be partially due to modulation of cecal microflora. It was also concluded that diet composition, mainly protein concentration, affects the responses of chickens to feed medication with ionophores plus growth-promotant antibiotics and vaccination with live oocysts of *Eimeria* spp. during a mixed coccidia infection.

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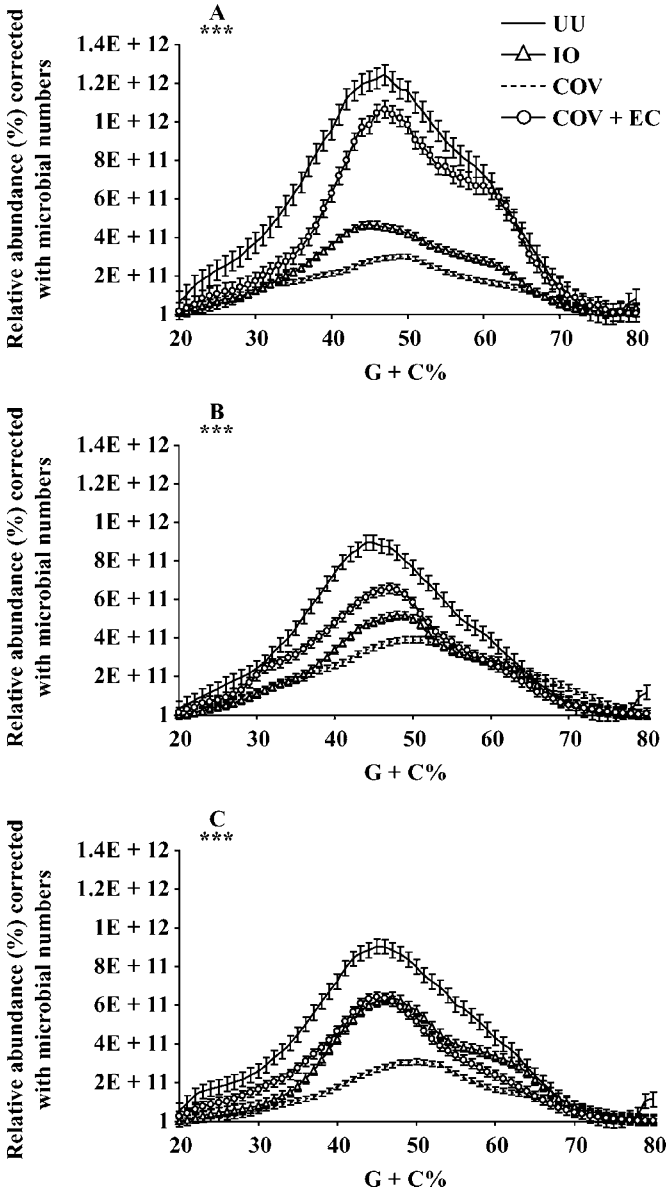

Figure 2. The G + C% profile of a cecal microbial community DNA-corrected with microbial numbers from 12 broilers per treatment. Treatments are compared within each dietary protein level: 19% (panel A), 21% (panel B), and 23% CP (panel C): UU = unmedicated-uninfected controls; IO = ionophore monensin; COV = coccidia vaccination; COV + EC = coccidia vaccination + dietary enzyme supplementation. ***$P \leq 0.001$.

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REFERENCES

Apajalahti, J. 2004. Microbial management: A new approach to development in animal nutrition. Proc. 5th Int. Congr. Feed Ind. Southern Africa, Sun City, South Africa. http://www.afma.co.za/AFMA_Template/feedpaper1.html Accessed Jan. 2005.

Apajalahti, J. H., A. Kettunen, M. R. Bedford, and W. E. Holben. 2001. Percent G+C profiling accurately reveals diet-related differences in the gastrointestinal microbial community of broiler chickens. Appl. Environ. Microbiol. 67:5656–5667.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult. Sci. J. 60:223–232.

Apajalahti, J., H. Kettunen, A. Kettunen, W. E. Holben, P. H. Nurminen, N. Rautonen, and M. Mutanen. 2002. Culture-independent microbial community analysis reveals that inulin in the diet primarily affects previously unknown bacteria in the mouse cecum. Appl. Environ. Microbiol. 68:4896–4995.

Bedford, M. R. 2000a. Exogenous enzymes in monogastric nutrition – their current value and future benefits. Anim. Feed Sci. Technol. 86:1–13.

Bedford, M. R. 2000b. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimise subsequent problems. World's Poult. Sci. J. 56:347–365.

Cebra, J. J. 1999. Influences of microbiota on intestinal immune system development. Am. J. Clin. Nutr. 69:1046S–1051S.

Chapman, H. D., T. E. Cherry, H. D. Danforth, G. Richards, M. W. Shirley, and R. B. Williams. 2002. Sustainable coccidiosis control in poultry production: The role of live vaccines. Int. J. Parasitol. 32:617–629.

Dalloul, R. A., and H. S. Lillehoj. 2005. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis. 49:1–8.

Dalloul, R. A., H. S. Lillehoj, T. A. Shellem, and J. A. Doerr. 2003. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. Poult. Sci. 82:62–66.

Drew, M. D., N. Syed, B. G. Goldade, B. Laarveld, and A. G. Van Kessel. 2004. Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens* in broiler chickens. Poult. Sci. 83:414–420.

Fernandez, F., R. Sharma, M. Hinton, and M. R. Bedford. 2000. Diet influences the colonisation of *Campylobacter jejuni* and distribution of mucin carbohydrates in the chick intestinal tract. Cell. Mol. Life Sci. 57:1793–1801.

Fukata, T., Y. Hadate, E. Baba, and A. Arakawa. 1991. Influence of bacteria on *Clostridium perfringens* infections in young chickens. Avian Dis. 35:224–227.

Hodgson, J. N. 1970. Coccidiosis: Oocyst-counting technique for coccidiostat evaluation. Exp. Parasitol. 28:99–102.

Hume, M. E., E. O. Oviedo-Rondón, C. Hernández, and S. Clemente-Hernández. 2006. Effects of feed additives and coccidia infection on microbial ecology of broilers. Poult. Sci. 85:2106–2111.

Idris, A. B., D. I. Bounous, M. A. Goodwin, J. Brown, and E. A. Krushinskie. 1997. Quantitative pathology of small intestinal coccidiosis caused by *Eimeria maxima* in young broilers. Avian Pathol. 26:731–748.

Jiang, Q., P. W. Waldroup, and C. A. Fritts. 2005. Improving the utilization of diets low in crude protein for broiler chicken. 1. Evaluation of special amino acid supplementation to diets low in crude protein. Int. J. Poult. Sci. 4:115–122.

Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.

Kelly, D., and S. Conway. 2005. Bacterial modulation of mucosal innate immunity. Mol. Immunol. 42:895–901.

Kettunen, H. L., A. S. Kettunen, and N. E. Rautonen. 2003. Intestinal immune responses in wild-type and ApcMin/+ mouse, a model for colon cancer. Cancer Res. 63:5136–5142.

Kettunen, H., and Rautonen, N. 2005. With betaine and exogenous enzymes towards improved intestinal health and immunity, and better performance of broiler chicks. Poult. Sci. 84(Suppl. 1):47. (Abstr.)

Lillehoj, H. S., and E. P. Lillehoj. 2000. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis. 4:408–425.

Lundén, A., P. Thebo, S. Gunnarsson, P. Hooshmand-Rad, R. Tauson, and A. Uggla. 2000. *Eimeria* infections in litter-

based, high stocking density systems for loose-housed laying hens in Sweden. Br. Poult. Sci. 41:440–447.
McDougald, L. R. 2003. Coccidiosis. Pages 974–991 in Diseases of Poultry. 11th ed. Y. M. Saif, ed. Blackwell Publishing Co., Ames, IA.
NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
Oviedo-Rondón, E. O., M. E. Hume, C. Hernández, and S. Clemente-Hernández. 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poult. Sci. 85:854–860.
Persia, M. E., E. L. Young, P. L. Utterback, and C. M. Parsons. 2006. Effects of dietary ingredients and *Eimeria acervulina* infection on chick performance, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 85:48–55.
Richter, G., and J. Wiesner. 1988. Relation between the protein supply of chicks and disposition to *Eimeria tenella* infections. Arch. Exp. Veterinarmed. 42:147–153.
Sall, J., and A. Lehman. 1996. JMP Start Statistics: A Guide to Statistical and Data Analysis Using JMP and JMP IN Software. Duxbury Press, Eadsworth Publishing Co., Belmont, CA.
SAS Institute. 2001. SAS User's Guide. Version 8 ed. SAS Inst. Inc., Cary, NC.
Sharma, V. D., M. A. Fernando, and J. D. Summers. 1973. The effect of dietary crude protein level on intestinal and cecal coccidiosis in chicken. Can. J. Comp. Med. 37:195–199.
Si, J., C. A. Fritts, D. J. Burnham, and P. W. Waldroup. 2004. Extent to which crude protein may be reduced in corn-soybean meal broiler diets through amino acid supplementation. Int. J. Poult. Sci. 3:46–50.
Sohail, S. S., M. M. Bryant, D. A. Roland Sr., J. H. Apajalahti, and E. E. Pierson. 2003. Influence of Avizyme 1500 on performance of commercial leghorns. J. Appl. Poult. Res. 12:284–290.
Wages, D. P., and O. Kenneth. 2003. Necrotic enteritis. Pages 781–785 in Diseases of Poultry. 11th ed. Y. M. Saif, ed. Blackwell Publishing Co., Ames, IA.
Williams, R. B. 1998. Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int. J. Parasitol. 28:1089–1098.
Williams, R. B. 2002. Anticoccidial vaccines for broiler chickens: Pathways to success. Avian Pathol. 31:317–353.
Williams, R. B. 2003. Anticoccidial vaccination: The absence or reduction of numbers of endogenous parasites from gross lesion in immune chickens after virulent coccidial challenge. Avian Pathol. 32:535–543.
Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159–180.