Effects of Dietary Protein on Restrict-Fed Broiler Breeder Pullets During a Coccidial Challenge[1]

JILL E. YAISSLE,* TERESA Y. MORISHITA,† and MICHAEL S. LILBURN*,[3]

*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio 44691 and †Department of Veterinary Preventative Medicine, The Ohio State University, Columbus, Ohio 43210

ABSTRACT In each of two experiments with young, feed-restricted broiler breeder pullets, the effects of differences in dietary protein intake on intestinal development and growth were studied. All pullets were restrict-fed either a 15 or 19% CP diet to see whether differences in dietary protein would influence intestinal growth in the face of controlled exposure to coccidiosis. In each experiment, pullets were vaccinated with one of three dilutions of Coccivac® (control, 1X, 4X), each level representing a different proportion of the manufacturers' suggested dosage level. Experiment 1 was conducted in battery cages with wire floors, and no infection was established, most likely because of a lack of oocyst recycling. The pullets that were restrict-fed the 19% CP diet had a significantly heavier *Pectoralis major* breast muscle weight at 14 and 21 d postvaccination (PV) and heavier BW at 21 d PV. Experiment 2 was conducted in floor pens with litter. In this experiment, coccidiosis was successfully established as coccidial oocysts invaded the mucosal cells of the villi in the upper small intestine. Pullets fed the 19% CP diet had significantly heavier BW at 14, 28, and 35 d of age. There were, however, no significant effects caused by level of dietary protein or vaccination dose on intestinal development (villus height and crypt depth). In conclusion, mild coccidial infections induced *via* the administration of commercial anticoccidial vaccines do not warrant changes in dietary protein during the onset of feed restriction in young broiler breeder pullets.

(*Key words*: protein, coccidiosis, breast muscle, intestinal development, broiler breeders)

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INTRODUCTION

Avian enteric coccidiosis is a protozoan disease caused by species of *Eimeria*. It is a concern to the poultry industry due to the potential for morbidity and mortality (McDougald and Reid, 1991). The morbidity associated with the disease within the broiler breeder segment of the broiler industry has contributed to the increased use of modified live vaccines. Commercial live vaccines, *i.e.*, Coccivac®[3], consist of low numbers of infective oocysts from many *Eimeria* species (Long, 1993; Shirley, 1993). The vaccine, usually given within the first 10 to 14 d after hatch, allows immunity to slowly build through continual exposure to recycling oocysts.

It is widely recognized that the genetic progress evident in modern broiler strains is largely due to indirect selection for feed intake (Chambers *et al.*, 1981). The potential for rapid growth, however, is negatively correlated with reproductive potential (Pym and Dillon, 1974; McDaniel *et al.*, 1981; McDaniel, 1983; Siegel and Dunnington, 1985). The end result is the evolvement of physical feed restriction programs for parent stock broiler breeders, usually from 2 wk of age throughout the remainder of the productive life of the flock.

Many reports have studied the relationship between feed restriction and disease resistance (Zulkifli, *et al.*, 1993; Prajharaj, *et al.*, 1995; Nir *et al.*, 1996). It has been shown that compared with *ad libitum*-fed controls, pullets on feed restriction were less susceptible to a coccidial challenge with *Eimeria tenella* (Zulkifli *et al.*, 1993). There have also been a number of reports on the influence of dietary protein on the severity of the symptoms related to coccidiosis (Britton *et al.*, 1964; Harms *et al.*, 1967; Welch *et al.*, 1986; Ruff, 1993). In reports by Harms *et al.* (1967) and Welch *et al.* (1986), higher levels of dietary protein enabled birds to better cope with a coccidial challenge compared with birds fed lower protein diets. These results differ from the conclusions of Britton *et al.* (1964), who suggested that higher levels of dietary protein contributed to the severity of a coccidial infection after challenge.

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[2]To whom correspondence should be addressed: lilburn.1@osu.edu
[3]American Scientific Laboratories, Omaha, NE 68127.

Abbreviation Key: EOD = every-other-day; MLS = microscopic lesion scoring; PMAJ = *Pectoralis major* muscle; PV = postvaccination.

TABLE 1. Diet used in both experiments

Ingredients	Diet 15% CP	19% CP
Corn	69.00	64.20
SBM-48	16.50	22.00
Meat and Bone	—	4.50
Midds	8.85	5.00
DL-Meth	0.10	0.15
L-lysine	0.05	0.05
Others[1]	5.50	4.10
Analysis		
CP-Calculated	15.00	19.20
CP-Analyzed[2]	18.80	22.60
M.E. (kcal/kg)	2,904	2,912
Lysine	0.78	1.04
Methionine	0.33	0.43
TSAA	0.58	0.73

[1]Vitamins, trace minerals, and macrominerals.

[2]The analyzed CP is reported on a dry matter basis. The percentage DM in the 15 and 19% CP diets was 86 and 87%, respectively.

The objective of the present study was to study the interaction between dietary protein intake and the response to a coccidial challenge by restrict-fed broiler breeder pullets. Changes in growth, breast muscle weight, and intestinal development were the variables measured.

MATERIALS AND METHODS

Birds, Pens, and Diets

In Experiment 1, day-old broiler breeder pullets (n = 180) were randomly placed in 18 pens in two Petersime brooder batteries.[4] In Experiment 2, day-old broiler breeder pullets (n = 480) were placed in 24 floor pens with new litter. In both studies, pullets were given *ad libitum* access to a 19% CP starter diet (Table 1) until 7 d (Experiment 1) or 10 d of age (Experiment 2). At 7 or 10 d of age, respectively, half the pens were switched to a 15% CP grower diet or continued on the 19% CP starter diet and at this age, all pullets were placed on every-other-day (EOD) feed restriction.

Vaccine Mixtures

Coccivac® is a live coccidial vaccine consisting of a suspension of live *Eimeria* oocysts to stimulate an immune response. The recommended vaccine for broiler breeders is Coccivac-D®, which contains oocysts from eight species of coccidia: *E. tenella*, *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. praecox*, *E. mivati*, and *E. hagani*. The concentrated vaccine is packaged in vials, with each vial recommended for administration to 1,000 chicks. In both experiments, the vaccine was administered in the drinking water with the following dilutions: 1) one vial of Coccivac-D®:3,000 mL H_2O (4X); 2) 1,000 mL 4X mixture:3,000 mL H_2O (1X); and 3) Control, H_2O alone. All pens were water-restricted for 12 h prior to vaccine administration. The vaccine was administered in quantities of 1,000 mL in chick water jugs and distributed at a rate of 1 jug per 10 pullets. No additional water was given until the vaccine mixture was consumed, usually within 2 d after administration.

[4]Petersime Inc., Gettysburg, OH 45328.

Experimental Protocol

Experiment 1. All pens were placed on EOD feed restriction beginning at 7 d of age. The pullets were alotted 45 g of feed EOD until 21 d of age. From 21 through 28 d of age (trial end), all pullets were fed 68 g per bird EOD. The experiment was a 2×3 factorial arrangement of treatments with 2 levels of dietary protein and 3 levels of vaccine dose. This design resulted in three replicate pens per level of protein and vaccine dose (n = 10 pullets per pen). Vaccine mixtures were administered at 7 d of age to all pens. At 5, 7, 14, and 21 d postvaccination (PV), one pullet per pen was randomly selected, weighed, and euthanatized with CO_2 gas. The right half of the *Pectoralis major* (PMAJ) muscle was dissected and weighed. The upper small intestine including the duodenum (1 cm from the junction with the gizzard) and jejunem (1 cm proximal to the yolk stalk) was removed and fixed in 10% neutral buffered formalin. The experiment lasted 28 d and the pullets were exposed to continuous light for the entire study.

Experiment 2. All pens were placed on EOD feed restriction at 10 d of age. The pullets were initially given 55 g feed per bird EOD at the onset of restriction; this was increased to 78 g per bird EOD at 21 d of age. Average pen weights were recorded at 14, 21, 28, and 35 d of age. This study was also a 2×3 factorial arrangement of treatments, similar to that described for Experiment 1. There were four replicate pens per level of dietary protein and vaccine dose (n = 20 birds per pen). Vaccine doses were similar to those described for Experiment 1 and were administered at 4 d of age. At 5, 7, 10, 14, and 28 d PV, two pullets per pen were randomly selected, individually weighed, and euthanatized with CO_2 gas. The right half of the PMAJ muscle from each bird was dissected and weighed. One pullet per pen was randomly selected, an intestinal section was collected, and the data collected as in Experiment 1.

Histology and Lesion Scoring

Intestinal sections were taken from birds at 5 and 14 d PV from the ascending duodenum and jejunum (2 cm proximal to the yolk stalk). The sections were processed, embedded in paraffin, sectioned at 5 μm, and stained with periodic Schiff (PAS) stain. Duodenal sections from 5 d PV were scored using the microscopic lesion scoring (MLS) method described by Idris *et al.* (1997). In this scoring method, four arbitrary microscopic fields are assigned a distribution score of 1 to 4 (based on the number of fields in which parasitic infection is present) and a severity score of 1 to 4 (based on the percentage of the villi that is parasitized by *Eimeria*). Severity scores were as follows: 0 = no *Eimeria* present; 1 = < 25% of the villi parasitized; 2 = 25

TABLE 2. Effects of dietary protein and coccidial vaccine dose on BW and *Pectoralis major* weights of broiler breeder pullets at 5, 7, 14, and 21 d postvaccination (Experiment 1)

	BW[1]				PMAJ			
	Days post-vaccination				Days postvaccination			
Treatment[2]	5	7	14	21	5	7	14	21
	(g)							
Control[3]	179.47	199.07	314.58	341.74	4.82	7.16	8.10	11.73
IX[4]	171.50	185.45	291.37	348.98	4.34	6.60	7.77	11.10
4X[5]	166.82	196.03	321.62	350.41	4.51	7.42	8.47	11.31
SEM	9.52	9.45	9.68	8.43	0.46	0.59	0.44	0.41
Diet[6]								
15% CP	171.66	194.39	302.87	332.18	4.52	6.60	7.40	10.44
19% CP	173.53	192.64	316.18	361.91	4.59	7.52	8.82	12.31
SEM	7.78	7.71	7.90	6.88	0.38	0.48	0.36	0.34
	P							
Source of variation								
Vaccine dose	0.65	0.58	0.12	0.74	0.76	0.62	0.55	0.54
Protein level	0.87	0.88	0.26	0.0032	0.91	0.20	0.0164	0.0002
Vaccine × protein	0.42	0.76	0.21	0.81	0.57	0.97	0.25	0.85

[1]Measurements from birds sampled.
[2]Average weight of eight birds per group.
[3]Sham dose of H_2O.
[4]1,000 mL 4X mixture:3,000 mL H_2O.
[5]One vial of Coccivac-D®:3,000 mL H_2O.
[6]Average weight of 24 birds.

to 50% of the villi parasitized; 3 = 50 to 75% of the villi parasitized; 4 = > 75% of the villi parasitized. These scores were combined to give a total lesion score of between 0 and 8. A mild infection would have a total score between 0.5 and 4. Measurements of villi in the jejunum sections from 14 d PV were made from images obtained from an Olympus IX70 inverted microscope[5]. The images were processed with a Vay Tek deconvolution imaging system[6] and measurements were made using Image-Pro Plus software[7]. Villus height (villus tip to crypt junction) and crypt depth (depth of the invagination between adjacent villus) were determined on 10 villi per bird.

Statistical Analysis

Data in both experiments were analyzed by analysis of variance using the General Linear Models (GLM) procedure of SAS® (1990). In both experiments, the main effects were protein level, vaccine dose, and the interaction of protein level by vaccine dose. Results were considered significant at $P \leq 0.05$.

RESULTS

Experiment 1

Clinical signs of coccidiosis, *i.e.*, diarrhea and weight loss, were not observed in any of the pullets and no lesions were observed upon histological examination. As duodenal sections did not contain any visible or histological lesions, jejunal histological measurements were not made. There were no significant differences in BW or PMAJ muscle weight due to vaccination dose (Table 2). There was an increase in BW at 21 d PV and increased PMAJ muscle weights at 14 and 21 d PV in pullets fed the 19% CP diet compared with those fed the 15% CP diet.

Experiment 2

Similar to the results of Experiment 1, there were no clinical signs of coccidiosis in Experiment 2. Upon necropsy

[5]Olympus America, Inc., Melville, NY 11757.
[6]Vay Tek, Fairfield, IA 52556.
[7]Media Cybernetics, Silver Springs, MD 20910.

TABLE 3. Villus height and crypt depth of broiler breeder pullets at 14 d postvaccination given different doses of a coccidial vaccine and fed a 15 or 19% CP diet (Experiment 2)

Vaccine dose	Villus height	Crypt depth
	(μm)	
Control[2]	117.45	23.24
IX[3]	113.25	26.75
4X[4]	132.25	27.36
SEM	8.13	1.25
Diet		
15% CP	123.89	26.42
19% CP	118.20	25.12
SEM	6.64	1.10
	P	
Source of variation		
Vaccine dose	0.23	0.10
Protein level	0.54	0.42
Vaccine × protein	0.66	0.89

[1]Vaccination doses.
[2]Sham dose of H_2O.
[3]1,000 mL 4X mixture:3,000 mL H_2O.
[4]One vial of Coccivac-D®:3,000 mL H_2O.

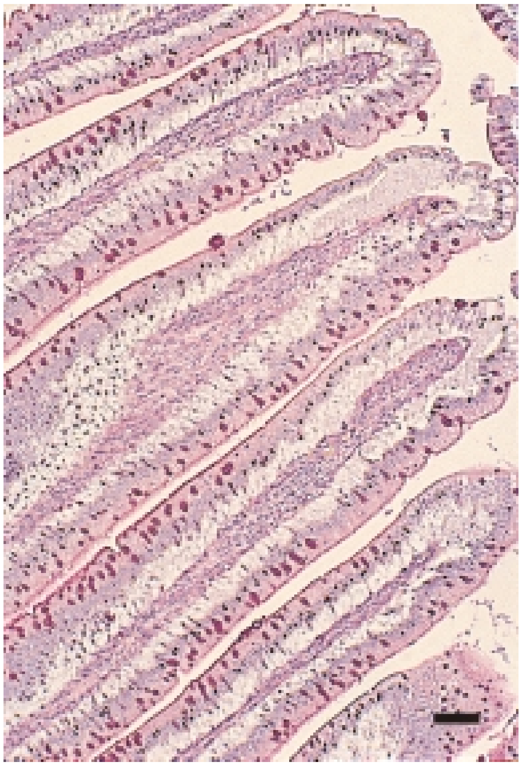

FIGURE 1. Light micrograph (100×) of a section of the ascending duodenum from a pullet in the control group at 5 d postvaccination. This micrograph represents a microscopic lesion score of 0. There was no evidence of *Eimeria* infiltration within the intestinal mucosa. Periodic acid Schiff, bar = 10 μm.

TABLE 4. Average BW per pen of broiler breeder pullets given coccidial vaccination and fed 15 or 19% CP diets at 14, 21, 28, and 35 d of age (Experiment 2)

	Average BW per pen			
	Days of age			
Vaccine dose	14	21	28	35
	(g)			
Control[2]	239.02	313.17	526.37	548.13
IX[3]	235.38	313.49	521.93	532.56
4X[4]	231.61	295.63	507.33	529.05
SEM	4.07	6.93	6.85	6.69
Diet				
15% CP	229.36	301.42	506.93	518.33
19% CP	241.32	313.43	530.16	554.83
SEM	3.32	5.66	5.60	5.46
	P			
Source of variation				
Vaccine dose	0.45	0.14	0.15	0.13
Protein level	0.0203	0.15	0.0088	0.0002
Vaccine × protein	0.55	0.86	0.71	0.56

[1]Vaccination doses.

[2]Sham dose of H_2O.

[3]1,000 mL 4X mixture:3,000 mL H_2O.

[4]One vial of Coccivac-D®:3,000 mL H_2O.

at 5 d and 14 d PV, however, white circular multifocal lesions, 1 to 2 mm, were present on the mucosal surface and visible from the serosal surface of the duodenum in some pullets given the 1X and 4X vaccine doses. At 5d and 14 d PV, respectively, 15 out of 16 and 6 out of 16 pullets had these lesions primarily in the ascending duodenum as visible from the serosal surface. Intestinal sections from Control birds fed both diets did not show any microscopic evidence of *Eimeria* infection (Figure 1). Intestinal sections from pullets in the 1X and 4X treatments fed both levels of dietary protein revealed oocysts, macrogametes, and microgametes infiltrating the mucosal cells lining the villi. Intestinal histopathology from pullets in the 1X treatment revealed parasitic infiltration involving just the tips of the villi (Figure 2), whereas sections from the 4X treatment revealed *Eimeria* infiltration along the length of the villi and in some cases down to the crypts (Figure 3).

The average total MLS scores (combination of the distribution and severity scores) for the 1X pullets were 1.88 and 0.38 for the 15% and 19% CP dietary treatments, respectively, and 3.25 and 3.31 for pullets in the 4X treatment. Duodenal sections taken at 5 d PV were used for lesion scoring, whereas jejunal sections taken at 14 d PV were used for crypt depth and villus height measures. Neither dietary protein level nor vaccine dose had any effect on these two intestinal measurements.

There were no significant effects of vaccine dose on average pen BW, but pullets fed the 19% CP diet were significantly heavier at 14, 28, and 35 d of age (Table 4). Only at 10 d PV, however, in the random sample of pullets selected for intestinal measures, were the pullets from the 19% CP treatment significantly heavier than those from the 15% CP treatment (Table 5). At 28 d PV, pullets sampled from the 1X treatment group were significantly heavier than the Control or 4X pullets, but there were no differences due to level of dietary protein. There were significant effects due to vaccine dose on PMAJ muscle weights at 7 and 14 d PV and significant effects due to dietary protein at 10 d PV. These differences were coincident with the vaccine dose and protein level effects on BW.

DISCUSSION

In Experiment 1, there were no clinical or histological signs of coccidiosis, which was probably a reflection of a lack of oocyst recycling due to the wire floors within the batteries. Throughout the course of this study, there was a consistent increase in BW in pullets fed the 19% CP diet together with a concomitant increase in the weight of the PMAJ breast muscle. At 14 and 21 d, muscle weights were 24 and 18% heavier in pullets fed the 19% CP *vs* 15% CP diets, respectively. This finding confirms earlier observations by Yaissle and Lilburn (1998) and Lilburn and Myers-Miller (1990) in which dietary protein effects on breast muscle weight were of greater magnitude than the observed differences in BW.

In Experiment 2, histopathology revealed *Eimeria* infiltration within the intestinal mucosa and the MLS scores confirmed that pullets in the 4X vaccine dose treatment had the most severe lesions. In birds fed *ad libitum*, coccidiosis can result in reduced performance (Adams *et al.*, 1996); lesions on the mucosal surface of the gastrointestinal tract,

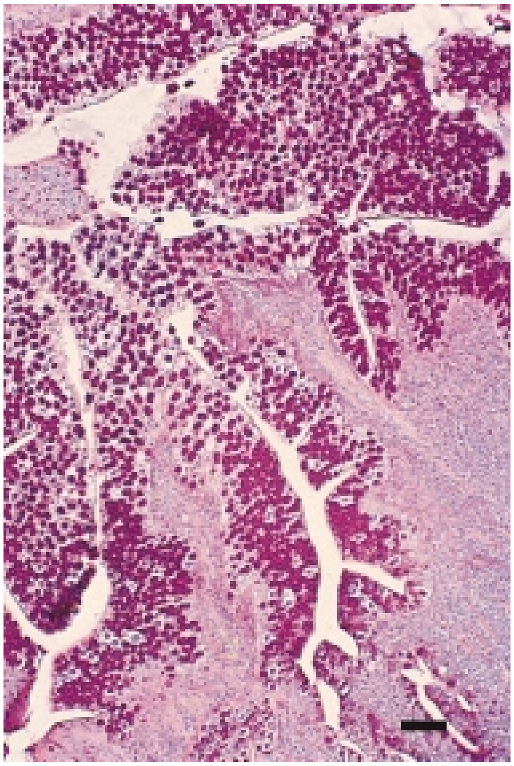

FIGURE 2. Light micrograph (100×) of a section of the ascending duodenum from a pullet in the 1× treatment group at 5 d postvaccination. This micrograph represents a microscopic lesion score of 1.5. Villus tips are infiltrated by oocysts and other *Eimeria* life-cycle stages. Periodic acid Schiff, bar = 10 μm.

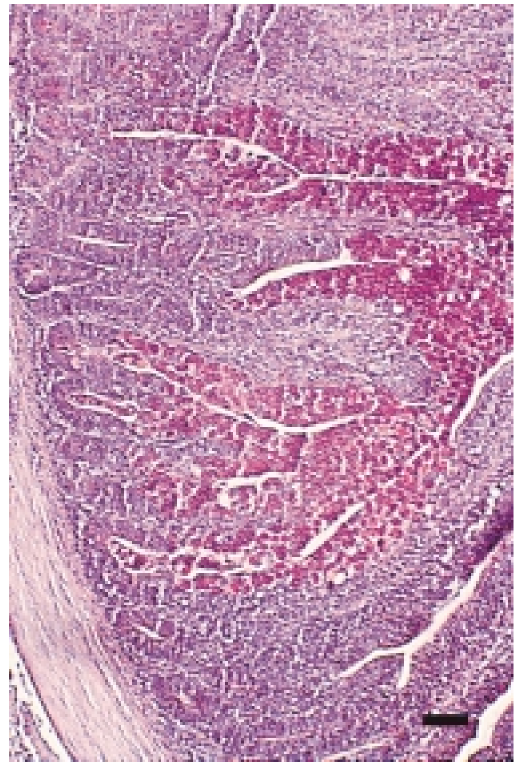

FIGURE 3. Light micrograph (100×) of a section of the ascending duodenum from a pullet in the 4× treatment group at 5 d postvaccination. This micrograph represents a microscopic lesion score of 5.25. Infiltration of oocyst and other *Eimeria* life-cycle stages extending far into the villi and down into the crypts. Periodic acid Schiff, bar = 10 μm.

TABLE 5. Effects of dietary protein and coccidial vaccination dose on BW and *Pectoralis major* weights of broiler breeder pullets at 5, 7, 10, 14 and 21 d postvaccination (Experiment 2)

	BW[1]					PMAJ				
	Days postvaccination					Days postvaccination				
Vaccine dose	5	7	10	14	28	5	7	10	14	28
	(g)									
Control[3]	138.53	180.30	265.80	312.33	599.98	3.13	4.21	7.35	9.69	17.28
IX[4]	139.05	193.39	228.61	295.36	625.19	3.09	5.25	6.02	8.27	18.40
4X[5]	136.84	173.93	242.19	283.74	560.81	2.95	4.21	6.80	7.27	16.56
SEM	4.87	6.68	11.01	8.32	16.31	0.18	0.26	0.43	0.36	0.69
Diet										
15% CP	140.02	178.48	231.88	294.71	599.32	3.16	4.38	6.20	8.16	16.75
19% CP	136.26	186.59	259.19	299.58	591.34	2.95	4.74	7.25	8.66	18.09
SEM	3.98	5.46	8.99	6.79	13.32	0.15	0.22	0.35	0.29	0.56
	P									
Source of variation										
Vaccine dose	0.95	0.12	0.08	0.06	0.0267	0.77	0.0101	0.12	0.0001	0.18
Protein level	0.51	0.30	0.0454	0.61	0.67	0.33	0.25	0.0478	0.23	0.09
Vaccine × protein	0.48	0.36	0.09	0.15	0.49	0.46	0.35	0.35	0.0055	0.21

[1]Measurements from birds sampled.

[2]Average weight of eight birds per group.

[3]Sham dose of H_2O.

[4]1,000 mL 4X mixture:3,000 mL H_2O.

[5]One vial of Coccivac-D®:3,000 mL H_2O.

[6]Average weight of 24 birds.

and bloody diarrhea (Ruff and Allen, 1990). In the present study, microscopic lesions were the only consistent indications of coccidial exposure. According to McDougald and Reid (1991), only a few areas of gross lesions are observed during a mild coccidial infection; in the present study, gross lesions were confined to the ascending dudodenum. Due to this observation and the lack of clinical signs, the level of pathology would be considered mild and thus would not have been severe enough to have influenced intestinal development (i.e. villus height, crypt depth). These latter measures of intestinal growth were also not affected by either protein level or vaccine dose.

Dietary protein retention has been reported to decrease during a coccidial challenge (Sharma and Fernando, 1975) and other studies have reported that feeding high levels of dietary protein enables birds to better cope with a coccidial challenge (Harms *et al.*, 1967; Welch *et al.*, 1986). In the latter two reports, however, the coccidial challenge was greater than that used in the present study and the birds were allowed *ad libitum* access to feed. In Experiment 2, no differences in BW due to vaccination dose were observed; however, pullets fed the 19% CP diet were significantly heavier at 14, 28, and 35 d of age. These results demonstrate that increased protein intake can have a positive effect on BW during feed restriction and a coccidial challenge.

In those pullets used for intestinal measurements, neither level of dietary protein nor vaccination dose had consistent effects on BW and PMAJ muscle weights; this was probably a reflection of our random sampling procedure combined with the inherent variability due to restricted feeding. At 14 d PV, there was an 8% increase in BW of control pullets compared with those in the 4X vaccine treatment ($P \leq 0.06$) *vs* a 33% increase in the weight of the PMAJ in the same pullets. This result suggests that during feed restriction, an induced environmental effect may have small nonsignificant effects on BW while having considerably larger effects on body composition, i.e. muscle weight.

In conclusion, in the present study, protein did have beneficial effects on BW even during a coccidial challenge. With respect to intestinal development or resistance to a mild coccidial challenge, there appears to be no significant benefit to increasing dietary protein levels in restrict-fed pullets, which suggests that the levels of dietary protein currently used within the industry will support active immunization and normal intestinal development after an anticoccidial vaccine challenge.

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