

Influence of coccidiosis vaccination on nutrient utilization of corn, soybean meal, and distillers dried grains with solubles in broilers

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ABSTRACT Two experiments were conducted to determine the impact of coccidiosis vaccination on the apparent ileal digestibility (AID) of nutrients and ileal digestible energy (IDE) in commonly used feed ingredients in broilers. Eight experimental treatments based on a factorial arrangement of coccidiosis vaccination (control with in-feed diclazuril [CTL] or vaccinated [VAC]) and 4 different diets were administered to male Cobb 500 broilers in floor pens containing 12 birds per pen. For the vaccinated group, a 3× dose of a live coccidiosis vaccine was given via oral gavage on the day of hatch. Experimental diets consisted of a basal diet and 3 test diets in which 30% of the basal diet was replaced with either corn, soybean meal (SBM), or distillers dried grains with solubles (DDGS) to allow for calculation of nutrient digestibility of individual ingredients by difference. Broilers were fed a common diet from 0 to 7 D and experimental diets from 7 to 12 D. On day 12, blood and ileal digesta were collected to measure plasma carotenoids and

determine AID of nitrogen, ether extract, IDE (experiments 1 and 2), and amino acids (AA) (experiment 2). Vaccination increased ($P < 0.05$) excreta oocyst counts and decreased ($P < 0.05$) plasma carotenoids when compared with CTL birds. Interactive effects ($P < 0.05$) were observed for AID of nitrogen (experiment 1) which was reduced by vaccination in birds fed the corn diet and increased for birds fed DDGS. No differences ($P > 0.05$) in IDE were observed between VAC and CTL birds in either experiment, whereas vaccination decreased ($P < 0.05$) AID of ether extract independently of diet. Interactive effects ($P < 0.05$) were observed for AA digestibility, whereby digestibility of all AA was reduced by VAC in corn diets but generally increased AA digestibility of DDGS diets, with minimal impact on SBM diets. In conclusion, the impact of coccidiosis vaccination on nutrient and energy digestibility varied among ingredients; however, digestibility was minimally impacted or improved with DDGS.

Key words: coccidiosis, digestibility, vaccination, amino acid, broiler

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INTRODUCTION

Accurate feed formulation requires knowledge of nutrient digestibility for each individual ingredient within the diet. Current widespread adoption of antibiotic-free production systems for commercial poultry has increased coccidiosis vaccination as a means of coccidiosis control, and the transient coccidiosis associated with live oocyst vaccination may negatively influence nutrient digestibility during the period of peak vaccine cycling. It is likely that any deleterious effects

of coccidiosis vaccination on nutrient digestibility may differ among individual feed ingredients, which would be important to recognize when formulating diets for vaccinated broilers. Indeed, Persia et al. (2006) subjected birds to an acute *Eimeria acervulina* infection at 9 D posthatch and found that amino acids (AA) digestibility and ME_n of diets containing fish meal were less impacted than those of diets only containing corn and soybean meal (SBM), indicating ingredient-dependent effects (Persia et al., 2006).

Previous research conducted in our laboratory demonstrated that nutrient digestibility for floor-reared broiler given a coccidiosis vaccine at the day of hatch was most impacted at 12 D posthatch (Gautier et al., 2020). It has also been shown that adjustments in digestible AA content to account for the predicted coccidiosis-induced reductions in AA digestibility improved the performance of broilers challenged with a 12× dose of a coccidial

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vaccine (Adedokun et al., 2016). Therefore, this suggests that an opportunity may exist for dietary adjustments to be made during the starter phase to support the performance of coccidiosis vaccinated broilers, and the effectiveness of such adjustments may be improved with knowledge of the impact of vaccination on nutrient digestibility of individual feed ingredients.

Current research to quantify the impact of a coccidiosis challenge on nutrient digestibility has primarily been based on the digestibility of the complete feed rather than for individual ingredients and has been conducted in battery cages where oocyst cycling is prevented. Furthermore, these models have generally involved a single inoculation with a large number of oocysts that greatly exceeds what would be ingested by the bird during typical vaccine cycling. Therefore, the objective of this experiment was to assess the impact of a coccidiosis vaccine challenge model on the nutrient utilization of corn, SBM, and distillers dried grains with solubles (DDGS) in floor-reared broilers. While corn and SBM are the primary feed ingredients for broiler diets in the United States, DDGS is an alternative ingredient that is compatible for use in all vegetable-based diets, which are commonly fed to coccidiosis-vaccinated broilers. Furthermore, the compositions of these ingredients vary widely in the amount and type of carbohydrates, protein, fiber, and lipids they contain, which could possibly provide insight into the class of nutrients that have the greatest influence on the response of broilers to coccidiosis vaccination.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

General Bird Husbandry and Dietary Treatments

Seven hundred and sixty-eight male broiler chicks were obtained from a Cobb 500 female line from a commercial hatchery on the day of hatch and allotted to 8 replicates pens (12 birds per pen; 0.09 m² per bird) of 8 treatments in a factorial arrangement of vaccination status and dietary treatment. As such, one-half (384) of the chicks were orally gavaged with a live oocyst vaccine (CocciVac-B52; Merck Animal Health, Intervet Inc., Millsboro, DE) at 3× the manufacturers recommended dose (VAC), whereas the other half were not vaccinated and received in-feed diclazuril (Clinacox; Huvepharma), a chemical coccidiostat, throughout the entire experiment (CTL). The vaccine contained 4 species of *Eimeria*, *E. mivati*, *Eimeria tenella*, *E. acervuline*, and *E. maxima*, and was delivered by oral gavage (0.25 mL/bird) using a stainless-steel gavage needle to provide uniform administration. All chicks were group-weighted and were distributed based on vaccine status (VAC vs. CTL) to 64 floor pens on unused pine shavings. Throughout the

trial, litter was sprayed daily with water to increase litter moisture to promote oocyst sporulation. Each floor pen was equipped with a hanging feeder and a nipple drinker line. Birds were provided access to feed and water *ad libitum* throughout the 12-day experiment, and the lighting schedule and temperature targets were adjusted according to management guidelines published by the primary breeder (Cobb-Vantress, 2015). Birds were provided a nutritionally complete common corn-SBM diet until 7 D of age. Experimental diets were fed from 7 to 12 D of age, and for all experimental diets, choline chloride, vitamins, and minerals were kept constant to prevent deficiencies of these nutrients.

At 7 D of age, CTL and VAC birds were weighed and provided 1 of 4 experimental diets that included the same diet as fed the previous 7 D (basal) or diets in which 30% of this basal diet was replaced with either corn, SBM, or DDGS to allow for the determination of apparent ileal digestibility (AID) of nutrients and ileal digestible energy (IDE) (experiments 1 and 2) and AID of AA (experiment 2) of these individual ingredients using the difference method (Table 1). The same sources of test corn, SBM, and DDGS were used in both experiments, and these samples were analyzed for protein, ether extract, and gross energy. The protein concentrations were 11.6, 48.1, and 31.0% for corn, SBM, and DDGS, respectively. Ether extract concentrations were 2.73, 1.60, and 8.89%, and gross energy concentrations were 3,795, 4,275, and 4,529 kcal/kg for corn, SBM, and DDGS (as-is basis), respectively.

Determination of Growth Performance, Excreta Oocyst Shedding, and Plasma Carotenoids

Although growth performance was not a primary objective of this trial, birds and feeders were weighed at 0, 7, and 12 D posthatch for calculation of body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) to assess growth performance as influenced by coccidiosis vaccination. All dead and culled birds were weighed individually, and FCR calculations were adjusted to include the weight gain of dead birds.

Oocyst shedding as indicated by the number of oocysts per gram of excreta samples collected from each pen was determined before bird placement and at 7 and 12 D after vaccination to ensure *Eimeria* infection. Samples of the unused shavings were taken before bird placement to confirm the absence of oocysts, and at 7 and 12 D, fresh excreta samples were collected from each pen using wax paper placed on the litter 12 h before collection. All samples were placed in airtight conical tubes and kept refrigerated until processing. Samples were soaked in water overnight and homogenized by vigorous stirring. After homogenization, 1 mL of sample was further diluted with 9 mL of saturated salt solution and pipetted into the chamber of a McMaster counting slide. Duplicate counts were made for each sample using the following equation:

Table 1. Composition of basal diet fed to broilers from 0 to 7 D and basal and experimental diets fed to broilers from 7 to 12 D posthatch.

Ingredient, % as-fed	Basal ¹	Experimental		
		Basal corn	Basal SBM	Basal DDGS
Corn	61.55	42.33	42.33	42.33
Soybean meal (46.3%)	31.75	21.85	21.85	21.85
Test corn	-	30.00	-	-
Test SBM	-	-	30.00	-
Test DDGS	-	-	-	30.00
Soybean oil	2.35	1.62	1.62	1.62
Limestone	0.99	0.99	0.99	0.99
Dicalcium phosphate	1.67	1.67	1.67	1.67
Salt	0.42	0.42	0.42	0.42
DL-methionine	0.25	0.17	0.17	0.17
L-lysine HCl	0.11	0.08	0.08	0.08
L-threonine	0.05	0.03	0.03	0.03
Trace mineral premix ²	0.10	0.10	0.10	0.10
Vitamin premix ³	0.10	0.10	0.10	0.10
Se premix ⁴ (0.06%)	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.05	0.05	0.05	0.05
Santoquin	0.02	0.02	0.02	0.02
Titanium dioxide	0.50	0.50	0.50	0.50
Inert filler ⁵	0.05	0.05	0.05	0.05
Calculated composition, % unless noted otherwise				
AME _n , kcal/kg	3,088	3,140	2,858	2,949
CP	19.50	15.61	27.29	21.45
Digestible lysine	1.11	0.83	1.61	0.97
Digestible TSAA	0.83	0.66	0.93	0.82
Calcium	0.86	0.83	0.92	0.85
Available P	0.44	0.41	0.49	0.51
Analyzed composition, % unless noted otherwise ⁵				
Experiment 1				
Gross energy, kcal/kg	3,938	3,885	3,952	3,954
CP	22.25	19.05	27.20	22.40
Ether extract	4.98	3.87	3.70	5.09
Experiment 2				
Gross energy, kcal/kg	3,989	3,960	4,097	4,212
CP	21.10	17.60	29.15	24.40
Ether extract	3.70	4.03	3.79	6.94

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL = control, birds were given an in-feed anticoccidial drug; DDGS, distillers dried grains with solubles; SBM, soybean meal; VAC = birds were given a 3 × dose of vaccine on 0 D.

¹All birds were fed the basal diet till 7 D of age. At 7 D of age, CTL and VAC birds were provided 1 of 4 experimental diets that included the same diet as fed the previous 7 D (basal) or experimental diets.

²Supplied the following per kg of diet: manganese, 100 mg; zinc, 100 mg; copper, 10.0 mg; iodine, 1.0 mg; iron, 50 mg; magnesium, 27 mg.

³Supplied the following per kg of diet: vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine, 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3.5 mg; biotin, 0.33 mg.

⁴Supplied 0.12 mg of selenium per kg of diet.

⁵For birds in the control group, Clinacox (Huvepharma Inc., Peachtree City, GA) provided 1 mg/kg diclazuril to the diet at the expense of sand which was used as the inert filler.

Oocysts per gram of excreta = (oocyst count × dilution × volume)/(volume of counting chamber × weight of sample), where the dilution was 10 and the volume of the counting chamber was 0.15 mL.

At 12 D posthatch, all birds were euthanized by CO₂ inhalation. Blood was collected from 2 randomly selected birds per pen via cardiac puncture and placed into tubes containing EDTA. After collection, tubes were placed on ice and subsequently centrifuged for 15 min at 1,300 × *g* and 4°C to separate plasma. Plasma from birds within a pen were pooled, aliquoted, and stored at -80°C until further analysis. All blood processing and carotenoid analysis procedures were conducted under yellow light and were determined by spectrophotometry previously described by Allen (1987).

Determination of Ingredient AID and IDE

At 12 D posthatch, ileal contents from all birds in each pen, including the 2 birds randomly selected for the collection of blood, were collected by gently flushing the distal half of the ileum using deionized water. Digesta samples within each pen were pooled and frozen (-20°C) until analysis. Frozen digesta samples were lyophilized and ground using an electric coffee grinder to provide an evenly ground sample while avoiding significant loss. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, and ether extract content. Gross energy was determined using a bomb calorimeter (Parr 6200 bomb calorimeter; Parr Instruments Co., Moline, IL.). Nitrogen

Table 2. Effects of coccidiosis vaccination on oocyst per gram of excreta sample (OPG) and plasma carotenoid concentration (µg/mL)¹.

Item	Basal		Basal corn		Basal SBM		Basal DDGS		P values ²		
	CTL	VAC	CTL	VAC	CTL	VAC	CTL	VAC	Diet	Vaccination	Interaction ³
Experiment 1											
OPG, day 7	2,182	4,736	-	-	-	-	-	-	N/A	0.001	N/A
OPG, day 12	756	2,724	2,275	7,786*	567	3,075*	790	2,789	0.001	0.001	0.040
Plasma carotenoids, day 12	1.42	0.83	1.52	1.00	1.48	0.97	1.62	0.74	0.942	0.003	0.838
Experiment 2											
OPG, day 7	1,871	40,490	-	-	-	-	-	-	N/A	0.001	N/A
OPG, day 12	2,187	21,678	4,398	22,650	2,028	22,488	5,220	21,409	0.840	0.001	0.583
Plasma carotenoids, day 12	1.90	1.46	1.91	1.46	1.66	1.01	1.75	1.46	0.712	0.060	0.961

Abbreviations: CTL, control, birds were given an in-feed anticoccidial drug; DDGS, distillers dried grains with solubles; SBM, soybean meal; VAC, birds were given a 3 × dose of vaccine on 0 D.

¹Values are LSMeans of 7 or 8 replicate pens.

²Overall ANOVA P values for the effects of diet, vaccination, and their interaction.

³In the case of an interaction, an asterisk (*) denotes statistical significance (P < 0.05) of change due to vaccination within a diet type.

was determined using the combustion method (Fisons NA-2000; CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006), and ether extract was determined according to AOAC (2006) method 920.39. Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO2 concentrations were determined in duplicate following the procedures of Short et al. (1996). AID of dry matter, gross energy, ether extract, and nitrogen were calculated using the following equation:

$$AID, \% = \left\{ \left[(X/TiO_2)_{diet} - (X/TiO_2)_{digesta} \right] / (X/TiO_2)_{diet} \right\} \times 100,$$

where (X/TiO₂) = ratio of nutrient concentration (%) to TiO₂ (%) in the diet or ileal digesta. Energy digestibility (%) values obtained from this equation were multiplied by the gross energy content of the feed to calculate apparent IDE in units of kcal/kg. In experiment 2, diets and digesta were sent to a commercial laboratory (University of Missouri Agricultural Experiment Station Chemical Laboratory, Columbia, MO) for determination of total AA content (methods 982.30 E (a,b,c) and 985.28; AOAC International, 2006) for determination of AID of AA.

Upon determination of AID of nutrients and IDE for complete experimental diets, these values were calculated for the individual test ingredients using the following equation:

$$AID \text{ of ingredient, } \% = [AID(\%) \text{ of test ingredient diet}] - [AID(\%) \text{ of basal diet} \times 0.66] / 0.30,$$

where 0.66 was the proportion of the basal diet and 0.30 was the proportion of the test ingredient. The remaining 4% of the diet was the portion kept constant to prevent nutrient deficiencies as described previously.

Statistical Analysis

Treatments were comprised of a factorial arrangement of vaccination status (CTL or VAC) × 4 diet types in a completely randomized design. Pen was considered the experimental unit with 8 replicate floor pens per treatment. Data on individual ingredients were analyzed as a factorial arrangement of vaccination status (CTL or VAC) × 3 ingredients. Data were subjected to a 2-way ANOVA using the MIXED procedure of SAS 9.4 to assess the main effects of dietary treatment, vaccination status, and their interaction. Statistically different treatment means were separated using a Tukey's multiple comparison test, and orthogonal contrasts between CTL and VAC birds were used to assess the impact of vaccination on birds within a dietary treatment. Statistical significance was considered at P < 0.05.

Table 3. Effects of coccidiosis vaccination on the apparent ileal digestibility of nutrients (%) and energy (kcal/kg) of experimental diets in broilers at 12 D posthatch¹.

Item	Basal		Basal corn		Basal SBM		Basal DDGS		SEM	P values ²		
	CTL	VAC	CTL	VAC	CTL	VAC	CTL	VAC		Diet	Vaccination	Interaction ³
Experiment 1												
Dry matter	64.6	67.0	75.6	72.1*	60.4	60.9	65.6	64.0	0.95	0.001	0.369	0.011
Nitrogen	74.6	77.8*	82.5	77.7*	73.4	73.4	74.1	79.8*	0.88	0.001	0.125	0.001
Ether extract	74.4	63.6	71.3	59.5	82.9	78.1	72.4	72.4	3.07	0.001	0.002	0.157
IDE	3,009	3,134	3,383	3,378	2,830	2,819	2,976	2,854	47	0.001	0.918	0.062
Experiment 2												
Dry matter	79.2	76.8	73.5	70.4	65.7	64.7	69.0	68.2	1.37	0.001	0.054	0.765
Nitrogen	86.6	84.5	79.8	77.8	80.7	78.5	78.8	80.1	1.01	0.001	0.082	0.248
Ether extract	76.4	67.1	67.0	55.6	77.3	73.4	76.4	72.5	2.30	0.001	0.001	0.258
IDE	3,483	3,409	3,219	3,064	3,042	2,937	3,143	3,127	73	0.001	0.077	0.779

Abbreviations: CTL, control, birds were given an in-feed anticoccidial drug; DDGS, distillers dried grains with solubles; IDE, ileal digestible energy; SBM, soybean meal; VAC, birds were given a 3 × dose of vaccine on 0 D.

¹Values are LSM means of 8 replicate pens.

²Overall ANOVA P values for the effects of diet, vaccination, and their interaction.

³In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

RESULTS

Oocyst Shedding, Plasma Carotenoids, and Growth Performance

In both experiments, no oocysts were detected in the shavings before bird placement (data not shown), and VAC birds had greater ($P < 0.05$) oocyst output at 7 D posthatch than CTL birds (Table 2). In experiment 1, a diet by vaccine interaction for 12 D oocyst output was observed, whereby vaccination increased ($P < 0.05$) excreta oocyst output in birds fed the corn and SBM diets and did not differ ($P > 0.05$) for birds fed the basal and DDGS diets. In experiment 2, oocyst output was influenced by vaccination status and was higher ($P < 0.05$) in VAC birds than CTL at 12 D posthatch. Plasma carotenoid concentrations were lower ($P < 0.05$) and tended to be lower ($P = 0.06$) in VAC birds than in CTL birds in experiments 1 and 2, respectively, with no independent or interactive diet effects.

Birds and feed were weighed in both experiments to provide a general assessment of the impact of vaccination (data not shown). At 7 D posthatch, when all birds

had been fed the common corn-SBM diet and experimental diets had not yet been provided, broiler performance was impacted by vaccination. In both experiments 1 and 2, VAC birds had a 9% reduction ($P < 0.05$) in BWG when compared with CTL birds. In experiment 1, the reduction in BWG of VAC birds resulted in a higher ($P < 0.05$) FCR than CTL birds, with no differences ($P > 0.05$) in FI. Conversely, in experiment 2, VAC birds had a lower ($P < 0.05$) FI than CTL birds, with no differences ($P > 0.05$) in FCR.

Apparent Ileal Digestibility of Nutrients and IDE

Complete experimental diets The AID of nutrients and IDE of the complete diets for both experiments 1 and 2 are presented in Table 3. In experiment 1, a diet by vaccine interaction for AID of dry matter was observed, whereby vaccination reduced ($P < 0.05$) AID of dry matter in birds fed the corn diet but not those fed the basal, SBM, or DDGS diets. A diet by vaccine interaction on AID of nitrogen was also observed, where

Table 4. Effects of coccidiosis vaccination on the apparent ileal digestibility of nutrients (%) and energy (kcal/kg) of individual feed ingredients in broilers at 12 D posthatch¹.

Item	Corn		SBM		DDGS		SEM	P values ²		
	CTL	VAC	CTL	VAC	CTL	VAC		Ingredient	Vaccination	Interaction ³
Experiment 1										
Dry matter	109.6	91.8	59.2	55.4	76.4	65.8	3.19	0.001	0.001	0.082
Nitrogen	111.5	88.2*	81.0	74.3	82.8	95.4*	3.05	0.001	0.025	0.001
Ether extract	83.2	58.4*	112.0	120.2	77.3	112.9*	7.88	0.001	0.310	0.001
IDE	4,553	4,410	2,787	2,647	3,278	2,721	163	0.001	0.036	0.335
Experiment 2										
Dry matter	70.6	65.7	56.8	46.9	55.5	58.3	4.19	0.001	0.166	0.201
Nitrogen	77.3	74.9	78.5	74.3	72.0	79.5	3.45	0.982	0.916	0.201
Ether extract	82.5	42.7*	101.5	103.0	86.5	100.0	7.67	0.001	0.147	0.001
IDE	3,255	2,748	2,785	2,132	2,817	2,764	211	0.016	0.007	0.211

Abbreviations: CTL, control, birds were given an in-feed anticoccidial drug; DDGS, distillers dried grains with solubles; IDE, ileal digestible energy; SBM, soybean meal; VAC, birds were given a 3 × dose of vaccine on 0 D.

¹Values are LSM means of 7 or 8 replicate pens.

²Overall ANOVA P values for the effects of diet, vaccination, and their interaction.

³In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

AID of nitrogen was reduced ($P < 0.05$) by vaccination in birds fed the corn diet, increased ($P < 0.05$) by vaccination in birds fed the basal or DDGS diet, and was not influenced by vaccination ($P > 0.05$) in birds fed the SBM diets. Independent effects of vaccine status ($P < 0.05$) and diet type ($P < 0.05$) were observed for AID of ether extract. Vaccinated birds had a lower AID of ether extract than CTL birds, and AID of ether extract was highest ($P < 0.05$) for the SBM diet (81%) and lower and similar ($P > 0.05$) among the basal (69%), corn (65%), and DDGS (73%) diets. Diet type influenced IDE ($P < 0.05$) and was highest for the corn diet, intermediate for the basal diet, and lowest for the DDGS and SBM diets. Furthermore, interactive effects between diet type and vaccination status tended ($P = 0.062$) to influence IDE, where IDE tended to be reduced by vaccination in birds fed the DDGS diet, increased by vaccination in birds fed the basal diet, and did not differ ($P > 0.05$) in birds fed the corn or SBM diets.

In experiment 2, AID of dry matter was influenced by diet type ($P < 0.05$) and was highest for the basal diet (78%), intermediate for the corn (72%) and DDGS (69%) diets, and lowest for the SBM diet (65%). Furthermore, AID of dry matter tended ($P = 0.054$) to be lower in VAC birds than in CTL birds. Diet type influenced AID of nitrogen ($P < 0.05$), which was highest for the basal diet (86%) and not different ($P > 0.05$) among the corn (79%), SBM (80%), and DDGS (79%) diets. Independent effects of vaccine status ($P < 0.05$) and diet type ($P < 0.05$) were observed for AID of ether extract. Vaccinated birds had a lower AID of ether extract than CTL birds, and for diet type, AID of ether extract was lowest for the corn diet (62%) with no differences ($P > 0.05$) among the basal (72%), SBM (75%), and DDGS (75%) diets. Diet type influenced IDE

($P < 0.05$), which was highest for the basal diet, with no differences ($P > 0.05$) observed among the other 3 diet type, and IDE tended ($P = 0.077$) to be lower in VAC birds than in CTL birds.

Individual Feed Ingredients Regarding the digestibility of individual feed ingredients, there were several cases where the digestibility values of the test ingredient diets (corn, SBM, and DDGS) were much higher than those determined for the basal diets. Therefore, when the digestibility of individual feed ingredients was determined by the difference method, it led to digestibility values that were close to or exceeded 100% (Table 4). This indicates a potential lack of additivity due to nutrient interactions among ingredients in the basal and the test ingredient diets. In experiment 1, independent effects of vaccine status ($P < 0.05$) and ingredient ($P < 0.05$) were observed for AID of dry matter, where VAC birds had a lower AID of dry matter than CTL birds, and AID of dry matter was highest for corn (101%), intermediate for DDGS (71%), and lowest for SBM (57%). Ingredient by vaccine interactions ($P < 0.05$) for AID of nitrogen and ether extract were also observed, where both AID of nitrogen and ether extract were reduced ($P < 0.05$) by vaccination in birds fed corn, increased ($P < 0.05$) by vaccination in birds fed DDGS, and did not differ ($P > 0.05$) for birds fed SBM. Independent effects of vaccine status ($P < 0.05$) and ingredient ($P < 0.05$) were observed for IDE, where VAC birds had a reduction in IDE compared with CTL birds, and IDE was highest for corn, with no differences ($P > 0.05$) between SBM and DDGS.

In experiment 2, AID of dry matter was influenced by ingredient ($P < 0.05$), which was highest for corn (68%) and not different between SBM (52%) and DDGS (57%). Nitrogen digestibility ($P > 0.05$) was not influenced by

Table 5. Effects of coccidiosis vaccination on apparent ileal amino acid digestibility (%) of experimental diets at 12 D posthatch (exp. 2)¹.

Item	Basal		Basal corn		Basal SBM		Basal DDGS		SEM	<i>P</i> values ²		
	CTL	VAC	CTL	VAC	CTL	VAC	CTL	VAC		Diet	Vaccination	Interaction ³
Essential amino acids, %												
Arginine	92.0	90.9	88.4	87.1	86.9	87.0	86.0	86.7	0.49	0.001	0.253	0.113
Histidine	89.5	89.0	83.6	82.3	82.5	82.3	81.4	83.6	0.69	0.001	0.916	0.056
Isoleucine	87.9	86.5	81.9	80.3	80.3	79.8	79.5	81.9*	0.80	0.001	0.597	0.048
Leucine	88.6	87.1	83.5	81.8	80.3	79.5	81.1	84.3*	0.78	0.001	0.690	0.006
Lysine	90.0	89.0	84.5	82.9	83.5	83.4	81.6	81.9	0.71	0.001	0.191	0.511
Methionine	94.1	93.3	89.3	88.5	87.1	87.5	88.0	88.9	0.51	0.001	0.795	0.229
Phenylalanine	88.3	86.9	82.8	80.8	80.1	79.3	80.5	83.1*	0.80	0.001	0.453	0.017
Threonine	83.8	83.4	75.6	73.4	75.1	74.9	74.5	76.7	0.87	0.001	0.792	0.075
Valine	86.1	85.6	79.4	77.3	78.1	77.8	76.9	79.6*	0.84	0.001	0.875	0.032
Nonessential amino acids, %												
Alanine	88.1	87.7	82.9	81.1	79.9	79.4	80.3	83.1*	0.70	0.001	0.902	0.007
Aspartic acid	86.9	86.1	81.6	79.8	79.1	78.6	78.0	79.9	0.81	0.001	0.560	0.118
Cysteine	80.9	80.3	73.4	71.5	62.9	65.0	71.1	75.4*	1.14	0.001	0.202	0.029
Glutamic acid	91.6	90.6	87.9	86.6	85.8	85.0	84.9	86.7*	0.53	0.001	0.444	0.021
Glycine	85.5	84.9	77.6	76.1	76.0	76.3	75.0	77.6	0.83	0.001	0.762	0.074
Proline	88.1	86.6	83.0	81.3	79.1	79.3	80.1	83.7*	0.74	0.001	0.815	0.001
Serine	86.6	85.3	80.1	78.1	77.9	77.9	77.8	80.6*	0.82	0.001	0.801	0.016

Abbreviations: CTL, control, birds were given an in-feed anticoccidial drug; DDGS, distillers dried grains with solubles; SBM, soybean meal; VAC, birds were given a 3 × dose of vaccine on 0 D.

¹Values are LSM means of 7 or 8 replicate pens.

²Overall ANOVA *P* values for the effects of diet, vaccination, and their interaction.

³In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

Table 6. Effects of coccidiosis vaccination on apparent ileal amino acid digestibility (%) of individual feed ingredients at 12 D posthatch (exp. 2)¹.

Item	Corn		SBM		DDGS		SEM	<i>P</i> values ²		
	CTL	VAC	CTL	VAC	CTL	VAC		Diet	Vaccination	Interaction ³
Essential amino acids, %										
Arginine	93.9	90.0	89.6	89.6	86.1	88.9	1.59	0.020	0.776	0.102
Histidine	83.0	78.8	78.7	80.7	75.2	81.7*	2.15	0.493	0.395	0.041
Isoleucine	82.5	76.7	77.6	78.1	74.4	82.2*	2.29	0.696	0.637	0.014
Leucine	86.7	80.9	75.3	75.4	78.4	89.5*	2.20	0.001	0.297	0.001
Lysine	85.7	80.8	83.1	84.4	76.1	77.6	2.07	0.002	0.662	0.187
Methionine	91.7	89.1	84.9	87.4	87.9	90.4	1.73	0.043	0.540	0.219
Phenylalanine	84.3	78.3	76.1	76.4	77.5	86.3*	2.27	0.026	0.564	0.006
Threonine	68.7	60.6*	67.0	69.0	64.7	72.2*	2.56	0.241	0.812	0.009
Valine	76.0	68.8*	72.0	73.6	68.5	76.6*	2.48	0.986	0.665	0.009
Nonessential amino acids, %										
Alanine	83.3	77.3	72.9	73.5	74.4	84.6*	2.29	0.004	0.383	0.003
Aspartic acid	82.2	76.3	74.3	75.5	70.5	76.7	2.25	0.030	0.780	0.057
Cysteine	67.7	61.4	32.9	43.1*	60.7	74.5*	3.36	0.001	0.031	0.007
Glutamic acid	93.8	89.5*	85.9	85.5	82.7	89.4*	1.59	0.004	0.577	0.003
Glycine	72.5	67.3	67.1	70.0	63.6	71.5*	2.57	0.630	0.356	0.035
Proline	85.6	80.2*	73.6	76.4	76.5	87.8*	2.02	0.001	0.076	0.001
Serine	79.8	72.6*	72.4	74.9	71.9	81.1*	2.25	0.364	0.400	0.002

Abbreviations: CTL, control, birds were given an in-feed anticoccidial drug; DDGS, distillers dried grains with solubles; SBM, soybean meal; VAC, birds were given a 3 × dose of vaccine on 0 D.

¹Values are LSM means of 7 or 8 replicate pens.

²Overall vANOVA *P* values for the effects of diet, vaccination, and their interaction.

³In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

ingredient, vaccination status, or their interaction. An ingredient by vaccine interaction for AID of ether extract was observed, whereby vaccination reduced ($P < 0.05$) AID of ether extract in corn but not in SBM or DDGS. Similar to experiment 1, independent effects of vaccine status ($P < 0.05$) and ingredient ($P < 0.05$) were observed for IDE, where VAC birds had a reduction in IDE compared with CTL birds, and IDE was highest for corn, intermediate for DDGS, and lowest for SBM.

Apparent Ileal Amino Acid Digestibility (Experiment 2)

Complete experimental diets The experimental diet AA digestibility values determined in experiment 2 are presented in Table 5. Main effects ($P < 0.05$) of diet type on arginine, histidine, lysine, methionine, threonine, aspartic acid, and glycine were observed, and digestibility values of these AA were generally highest for the basal diet. Interactive effects between diet type and vaccination status ($P < 0.05$) were observed for AID of isoleucine, leucine, phenylalanine, valine, alanine, cysteine, glutamic acid, proline, and serine. For each of these AA, this interaction was due to an increase in AID with vaccination in birds fed DDGS, with no effect ($P > 0.05$) of vaccination in birds fed the basal, corn, or SBM diets.

Individual Feed Ingredients The individual feed ingredient AA digestibility values determined in experiment 2 are presented in Table 6. Main effects ($P < 0.05$) of ingredient on arginine, lysine, methionine, and aspartic acid were observed, and AID of these AA were

generally highest for corn. Interactive effects between ingredient and vaccination status ($P < 0.05$) were observed for AID of histidine, isoleucine, leucine, phenylalanine, threonine, valine, alanine, cysteine, glutamic acid, glycine, proline, and serine. For histidine, isoleucine, leucine, phenylalanine, alanine, and glycine, AID was increased by vaccination in birds fed DDGS, with no differences ($P > 0.05$) due to vaccination in birds fed corn or SBM. For threonine, valine, glutamic acid, proline, and serine, AID was increased by vaccination in birds fed DDGS, reduced by vaccination in birds fed corn, and did not differ ($P > 0.05$) with vaccination for birds fed SBM. The AID of cysteine was increased by vaccination in birds fed SBM and DDGS but did not differ ($P > 0.05$) for birds fed corn.

DISCUSSION

Estimates of nutrient digestibility for individual feed ingredients fed are greatly influenced by the assay method and associated diet types used, and the best approach may differ for the primary nutrient of interest. For example, it has been suggested that the use of semi-purified diets in which the test ingredient serves as the sole source of AA is the preferred method for generating AA digestibility values (Ravindran et al., 2017). Alternatively, the difference method is based on a practical diet replacement with the test ingredient of interest and digestibility values for individual test ingredients being calculated according their proportional contribution to the test diet (Kong and Adeola, 2013; Zhang and Adeola, 2017). This approach is often used for determination of ME_n of individual feed ingredients (Stefanello et al., 2016) but has also been used for

determination of AA digestibility (Fan and Sauer, 1995). To limit the number of experimental birds and owing to the potential drawbacks associated with semi-purified diets, such as reduced FI and enzyme secretions (Partridge et al., 1982; Shastak et al., 2014) and increased digesta passage rate (Colnago et al., 1984), which are all factors that could influence the desired development of coccidiosis induced by the live oocysts vaccine, a practical diet replacement assay was selected to simultaneously estimate the digestibility of nutrients, energy, and AA in the current experiment. Furthermore, focus of this experiment was to quantify any relative reductions in nutrient and energy digestibility due to vaccination that could be applied to any chosen set of digestibility values used by formulating nutritionists, rather than to establish actual digestibility values to be implemented in feed formulation.

As with the dietary approach, the impact of coccidiosis or coccidiasis on nutrient utilization will be influenced by the challenge model applied, with important factors including the species, number, and infectivity of the oocysts administered. The vaccine model used in the current experiment attempted to reflect conditions experienced by commercial broilers vaccinated for the control of coccidiosis. Therefore, a commercially-available coccidiosis vaccine, which contained a mixture of live *Eimeria* species, was administered to floor-reared birds at the day of hatch. As immunity to *Eimeria* is species specific, it is important to vaccinate birds against all *Eimeria* species that a commercial flock may encounter, rather than just a single species (Chapman et al., 2005). Furthermore, to ensure measurable and consistent impacts of this model, birds were orally gavaged with a dose of vaccinal oocysts that was higher (3×) than the manufacturer's recommended dose. Excreta oocyst output was increased in VAC birds at 7 D posthatch in both experiments and at 12 D posthatch in experiment 2, confirming that oocyst cycling occurred in the vaccinated birds. The reason for the greater increase in oocyst excretion with vaccination for birds fed the corn and SBM diets compared with those fed the basal and DDGS diet in experiment 1 is unknown, as this response was not observed in experiment 2. Previous research has demonstrated that trypsin promotes the excystation of sporozoites from sporulated *Eimeria* oocysts within the intestine (Britton et al., 1964), and therefore, increased oocyst shedding observed for VAC birds fed the SBM diet may have been attributed to an increase in pancreatic trypsin secretion due to the high dietary crude protein content. However, based on this explanation, the opposite would be expected for birds fed the corn diet, which was not the case. The reduction in plasma carotenoid concentrations in VAC birds was expected, as carotenoids are inversely related to oocyst output and have proven to be a sensitive indicator of coccidial-induced intestinal damage in birds (Holdsworth et al., 2004). Furthermore, carotenoids are fat-soluble components (Yonekura and Nagao, 2007), and these reductions were likely associated with the observed reductions in lipid digestibility discussed in the following sections. Although there was some variation between the 2 experiments, these

results collectively validate a successful vaccine challenge model in both experiments.

Owing to large differences observed in oocyst shedding between the 2 experiments, the impact on nutrient digestibility will be discussed individually for each experiment. In general, the complete diet nutrient digestibility values indicated that the effects of the vaccine challenge model varied with diet composition. In experiment 1, the reduction in AID of nitrogen observed in VAC birds fed the corn diet which was not observed for birds fed the other diets may have been associated with the increased oocyst output that occurred at that time point as described previously. However, the lack of a more pronounced impact on birds fed the corn diet in experiment 2 may indicate that the effects on this dietary group in experiment 1 were not related to the diet composition *per se* but increased oocyst output due to some other factor. In experiment 1, VAC birds fed the SBM diet also had increased oocyst output; however, no vaccine-induced reduction in AID of nitrogen was observed and may be attributed to the high dietary crude protein content. Previous research has shown that increased dietary concentrations of crude protein or AA can be beneficial to broilers during coccidiosis by increasing the supply of AA needed for intestinal repair (Parker et al., 2007; Lehman et al., 2009; Lee et al., 2011; Adedokun et al., 2016; Cloft et al., 2019). Interestingly, vaccination actually increased AID of nitrogen in birds fed the DDGS diets in experiment 1, and in experiment 2, vaccination increased the AID of 4 essential and 5 nonessential AA in birds fed the DDGS diets. Therefore, the impact of DDGS in broiler diets suggests additional mechanisms other than dietary crude protein content that may be beneficial for intestinal health during an enteric infection.

Vaccine-induced reductions in ether extract digestibility, independent of diet type, were consistently observed in both experiments, with AID ether extract values of 75.3 and 68.4 and 74.3 and 67.2% for CTL and VAC birds in experiments 1 and 2, respectively. Furthermore, the average reduction of 7.0 percentage units (9.3%) in AID of ether extract between the CTL and VAC birds was generally greater than the reduction for AID of DM, nitrogen, or AA. Other recent trials have also indicated that *Eimeria* exposure markedly impacts lipid digestibility (Amerah and Ravindran, 2015; Gautier et al., 2020). However, the vaccine-induced reductions in AID of ether extract did not translate to reduced IDE and may possibly be attributed to a lower vaccine impact on starch digestibility, which accounts for a much larger proportion of total dietary energy. Although not determined in the current experiment, previous results from our laboratory have shown that vaccination has only a moderate impact on AID of starch compared with ether extract. In both experiments herein, birds fed the basal and corn diets had a lower AID of ether extract but higher IDE values than birds fed the SBM and DDGS diets, further supporting that the reduction in AID of ether extract was not sufficient to markedly impact energy digestibility.

While the original objective of this experiment was to determine the digestibility of individual test ingredients (i.e., corn, SBM, and DDGS), relatively high IDE of corn in experiment 1 and digestibility values greater than 100% in both experiments 1 and 2 indicate a potential violation of the necessary assumption for the difference method that no interactions exist between the digestibility values of the test feed ingredient and basal diet components (Kong and Adeola, 2013). Glencross et al. (2004) also reported digestibility values in excess of 100% for a range of plant protein ingredients when the difference method was used and suggested it could be attributed to analytical error for nutrient analyses, poor mixing of the marker in the diet, a nonrepresentative diet sample, or interactions among ingredients. Nonetheless, relevant conclusions from the current experiment can be still drawn regarding the impact of the vaccine challenge on nutrient digestibility of the test ingredients.

The AA digestibility values of feed ingredients were generally similar to those reported in previous literature. The AID of lysine in SBM has been reported to range from 84 to 93% (Ravindran et al., 2005; Huang et al., 2006), slightly higher than the value of 83% obtained for CTL birds in the current experiment. The AID of lysine in corn has been reported to range from 79 to 86% (Huang et al., 2006; Adedokun et al., 2009), which falls within range for the value of 86% reported for CTL birds in the current experiment. Furthermore, the AID values of lysine are generally higher than those of threonine, which is in agreement with the results reported herein, as threonine is a major component of mucin production and continuous intestinal secretions likely attribute to low AID values (Fernandez et al., 1994). Threonine, as well as cysteine, serine, and proline, are major structural components of intestinal mucin (Montagne et al., 2004). The AID of several of these AA in corn, but not SBM or DDGS, were reduced by vaccination, indicating that, as expected, increased endogenous losses of these AA would have a proportionally higher impact on AID values of these AA in lower protein ingredients such as corn (Lemme et al., 2004).

The overall minimal impact of coccidiosis vaccination on nutrient digestibility, and even positive effect on AA digestibility in some cases, for birds fed DDGS may have been due to several factors. Distillers dried grains with solubles contain a high concentration of insoluble fiber (i.e., lignin, cellulose, hemicellulose) (Urriola et al., 2010), whereby insoluble fiber can decrease the viscosity of the digesta, increase digesta passage rate, and promote epithelial sloughing by physical contact through a process often referred to as the “scratch factor” for ruminants fed high-fiber material. This additional sloughing, while generally undesirable for nutrient utilization in nonruminant animals, may prevent the adhesion of *Eimeria* within the intestine and increase sloughing of parasitized enterocytes. Therefore, this may have increased the rate of recovery from the vaccine-induced infection for the DDGS-fed birds in the current

experiment, and it has been demonstrated that nutrient utilization of broilers after peak coccidial infection is very efficient (Fernando and McCraw, 1973; Turk, 1974; Ruff and Allen, 1982). Dietary DDGS also includes yeast, which is added during the fermentation process, and yeast cell wall components including mannan-oligosaccharides and β -glucans can modulate intestinal microflora, enhance immunocompetence, and increase nutrient digestibility (Ferket et al., 2002; Vohra et al., 2016). Perez et al. (2011) reported that including up to 20% DDGS in diets fed to broilers from 7 to 21 D of age, when birds were subjected to a severe *E. acervulina* infection at 10 D of age, did not positively or negatively affect the severity of an *Eimeria* infection on growth performance but did increase bacterial diversity in the cecum, which is generally considered beneficial to gastrointestinal health.

In summary, these results indicate that the effects of the vaccine challenge model varied among diet composition and thus the nutrient class. The vaccine challenge model had a marked impact on ether extract digestibility, with the greatest reduction observed for corn. Vaccinated birds fed SBM resulted in minimal impacts on overall nutrient digestibility, whereas vaccinated birds fed DDGS resulted in minimal impacts on ether extract digestibility, with improved nitrogen and AA digestibility. Although further research is needed, it appears that there is no reason to expect that DDGS of an acceptable quality would exacerbate, and actually may even benefit, intestinal function of broilers experiencing a mild coccidial infection.

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