ABSTRACT One-day-old broiler chicks (n = 300) were orally vaccinated (Coccivac-B) and divided into 6 groups to evaluate Arg at 3 levels of supplementation, 0, 0.3, or 0.6% [normal level (NARG), medium level (MARG), or high level (HARG), respectively], and 2 levels of vitamin E (VE), 40 or 80 IU/kg of feed (VE40 or VE80, respectively), in a factorial experiment. Birds were reared in floor pens with fresh pine shavings and provided a corn-soybean-based diet and water ad libitum. At d 14, all chickens were orally challenged with a mixture of Eimeria field isolates (Eimeria acervulina, Eimeria maxima, and Eimeria tenella). In vitro heterophil and monocyte oxidative burst (HOB and MOB, respectively) was measured at d 21 from cells isolated from peripheral blood. Antibody levels (IgG, IgM, and IgA isotypes, ELISA) and NO were measured at d 14 and 28. The HOB was lower in birds fed the VE40 diets but was increased with the MARG and HARG treatments, whereas birds fed the VE80 diet had a higher HOB irrespective of Arg level. Birds fed the VE80 diet had high levels of MOB, which was not further improved by Arg, whereas birds fed the VE40-MARG diet had the highest MOB response. Plasma NO was not affected by diet at d 14, but at d 28, plasma NO was higher in birds fed the VE80-MARG or the VE40-NARG diet and lower in birds fed the VE80-NARG or the VE40-MARG diet. Birds fed the VE40-HARG or VE80-MARG diet had the highest IgG levels at d 14, but at d 28, birds fed the VE80-MARG diet had the highest IgG levels. The IgM concentration was lower in birds fed NARG levels irrespective of VE levels at d 14, but at d 28, IgM levels were higher in birds fed the VE40-HARG or the VE80-MARG feed. The IgA concentration was not consistently affected at d 14 or 28. These results suggest that Arg and VE fed at levels higher than those recommended by the NRC may play complementary roles on the innate and humoral immune response against an Eimeria challenge, potentially improving vaccine efficacy and response to field infections.

Key words: arginine, vitamin E, coccidiosis, immune response

INTRODUCTION

Avian coccidiosis is one of the most common and important diseases in poultry production caused by protozoan parasites of the Eimeria genus (Chapman, 2001; McDougald, 2003; Dalloul and Lillehoj, 2005; Marien and de Gussem, 2007). Nine species of Eimeria have been described in chickens (Charlton et al., 2000); however, McDougald (2003) reported that only 7 species are pathogenic in chickens: Eimeria acervulina, Eimeria tenella, Eimeria mitis, Eimeria praecox, Eimeria maxima, Eimeria necatrix, and Eimeria brunetti. Each species produces separate and distinct recognizable symptoms, but in general, the parasites proliferate in the gut, subsequently producing lesions. This causes destruction of the epithelial cells and trauma to the intestinal mucosa and submucosa, which generates an interruption of nutrient absorption, dehydration, blood loss, and amplified susceptibility to other disease agents. Morbidity and mortality due to coccidial infections are usually proportional to the number of sporulated coccidial oocysts ingested and the immune status of birds (Charlton et al., 2000; McDougald, 2003).

A common method of coccidiosis control in commercial broilers has been the use of in-feed anticoccidial drugs (Chapman, 2001), but the appearance of Eimeria strains resistant to commonly used anticoccidials (Peek and Landman, 2003) has increased the use of commercially available coccidiosis vaccines (Vermeulen et al., 2010).
2001). In fact, the only practical alternative to avoid the use of anticoccidial drugs in poultry is to rely upon live vaccines (Chapman et al., 2002). Administration of live oocyst vaccines against coccidiosis with 3 to 4 species may not protect the flock against other species; on the other hand, live vaccines with more species may introduce unexpected pathogens. Thus, the lack of efficient vaccination procedures means that coccidiosis control remains largely dependent on routine use of anticoccidial drugs (Dalloul and Lillehoj, 2005).

Nutrition plays an important role in the proper immune function of chickens. Vitamin E (VE) and Arg, an essential amino acid in birds, have been reported to boost and modulate immune function under several disease challenges, including coccidiosis. Allen (1999) reported that either single or dual daily doses of Arg (500 mg/kg of BW) reduced oocyst shedding of *E. tenella* without affecting BW gain, plasma NO$_2^-$ + NO$_3^-$ levels, or lesion scores. In another study, chickens were experimentally inoculated with a range of doses of *E. acervulina* and fed with 1.64% Arg; birds infected with the highest oocyst doses had the lowest BW gains and the lowest plasma Arg and carotenoid concentration but showed the highest plasma NO$_2^-$ + NO$_3^-$ levels and the highest lesion scores (Allen and Fetterer, 2000). These results suggest that higher oocyst doses of *E. acervulina* reduce BW due to higher plasma NO$_2^-$ + NO$_3^-$ levels related with inflammatory processes during the immune response; moreover, the higher lesion scores and lower plasma Arg and carotenoid concentration may be associated with malabsorption due to damage of the epithelial mucosa. The effects of VE after *Eimeria* challenges have been controversial. Colnago et al. (1984) showed that chickens immunized with sporulated oocysts from *E. tenella* had better BW after challenge when fed with 100 IU of VE/kg of feed compared with birds fed a non-VE-supplemented corn-soybean meal-based diet, suggesting that VE boosts the immune response after *Eimeria* immunization. Conversely, Allen and Fetterer (2002) reported that a mild infection with *E. maxima* in birds fed diets with 153 IU of VE/kg of feed did not improve either BW gain or feed conversion ratio but augmented plasma α-tocopherol concentration and oocyst shedding compared with birds fed diets with 13.2 IU of VE/kg of feed; furthermore, severe *E. maxima* infection in birds fed diets with 200 IU of VE/kg of feed had lower BW gains, without affecting either feed conversion ratio or oocyst shedding, compared with birds fed diets with 13 IU of VE/kg of feed.

The previous reports suggest that Arg and VE, used alone, could enhance the immune response and improve the health of birds when exposed to *Eimeria*, although the results are not very consistent. Abdulkalykova et al. (2008) found that the combination of high levels of Arg (2.2%) and VE (80 IU/kg of feed) increased the amount of T and B cells and the CD4+ and CD8+ T-cell subpopulations after vaccination against infectious bursal disease virus, suggesting a synergistic effect between Arg and VE to improve the immune response. Thus, we hypothesize that the concurrent use of Arg and VE could enhance the immune response after coccidiosis vaccination and reduce problems associated with coccidial infections in the field. The objectives of this experiment were to evaluate the effects of Arg and VE supplementation at levels higher than those recommended by the NRC (1994) on the humoral immune response after vaccination and after a challenge with a field mix of *Eimeria* oocysts and on the innate immune response [in vitro heterophil and monocyte oxidative burst (HOB and MOB, respectively)] and plasma levels of NO after the *Eimeria* challenge.

**MATERIALS AND METHODS**

**Birds and Experimental Design**

Three hundred 1-d-old broiler chicks (Cobb 500) were obtained from a local commercial hatchery and randomly divided into 6 groups of 50 birds each. Chicks were reared on fresh pine shavings following conventional brooding and lighting regimens. All birds were provided with a corn-soybean meal basal diet (free of antibiotic growth promoters and coccidiostats) formulated to meet or exceed all of the NRC (1994) requirements for broilers, including 23% CP, 3,199 kcal of ME/kg, 1.44% Arg, and 40 IU of VE/kg of feed (the supplementation level used by the poultry industry). Dietary treatments were formulated by supplementing a basal diet. Three levels of Arg were tested, normal level (NARG, no further supplementation), medium level (MARG, 0.3% Arg supplementation), or high level (HARG, 0.6% Arg supplementation), and 2 levels of VE, no further supplementation (VE40) or VE supplemented to reach 80 IU/kg of feed (VE80), resulting in a 3 × 2 factorial arrangement of treatments. All Arg additions to the basal diet were in the form of l-arginine HCl (Sigma-Aldrich, Milwaukee, WI) and VE in the form of dl-α-tocopheryl acetate (Animal Science Products Inc., Nacogdoches, TX). Water, supplied from a low-pressure nipple-drinking water system, and feed were offered on an ad libitum basis. All protocols were approved by the institutional animal care committee.

**Coccidiosis Vaccination and Challenge**

At 1 d of age, all chicks were vaccinated with a live *Eimeria* oocyst vaccine (Coccivac-B, Intervet/Schering-Plough Animal Health Corp., Millsboro, DE) using a spray cabinet; chicks were allowed to preen for 1 h before placement. At d 14, all chickens were challenged with a mixture of *Eimeria* field isolates mixed into the feed. Dilutions of coccidial inoculate were prepared to reach the following oocyst levels per bird: $1 \times 10^5$ *E. acervulina*, $6 \times 10^4$ *E. maxima*, and $4 \times 10^4$ *E. tenella*. All birds were deprived of feed for 5 h, the inoculum dilutions were mixed into 500 g of feed/group of birds, and the mixture was offered to the chickens. It was expected that each bird would ingest $2 \times 10^5$ oocysts of...
the mixture of *Eimeria* field isolates (*E. acervulina*, *E. maxima*, and *E. tenella*). Normal feeding was resumed when more than 90% of the *Eimeria*-mixed feed was consumed.

**ELISA**

Peripheral blood samples (n = 8 per treatment) were obtained by vein puncture and allowed to clot for 2 h at room temperature then centrifuged at 400 × g for 8 min at 4°C, and the sera were collected and stored at 4°C for 4 h, finally kept at −80°C until analyses. The samples were collected at 14 d postvaccination (PV) and at 14 d postinfection (PI). The serum samples were used to measure the concentrations of IgG, IgA, and IgM isotypes, using chicken IgG, IgA, or IgM ELISA quantitation kits (Bethyl Laboratories, Montgomery, TX; Schuijffel et al., 2005). Briefly, flat-bottomed microtiter plates were coated for 60 min with capture antibody (goat anti-chicken IgG-Fc or IgM or IgA affinity purified) and coating buffer (0.05 M carbonate-bicarbonate, pH 9.6). Plates were washed 3 times with wash solution (50 mM Tris-buffered saline, 0.14 M NaCl, 0.05% Tween 20, pH 8.0), and wells were incubated with blocking (postcoat) solution (50 mM Tris-buffered saline, 0.14 M NaCl, 1% BSA, pH 8.0) for 30 min then rinsed 3 times with wash solution. The calibrator (chicken reference serum) and sample-conjugate diluent (50 mM Tris-buffered saline, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0) were used to do standards, whereas serum samples, thawed at 4°C overnight, were diluted at 1:1,000 in the sample-conjugate diluent. Then, they were incubated in wells for 60 min and washed 5 times with wash solution. Detection antibody horseradish peroxidase (goat anti-chicken IgG-Fc or IgA or IgM) diluted in sample-conjugate diluent was added to wells, incubated for 60 min, and rinsed 5 times with wash solution. Enzyme substrate (3,3′,5,5′-tetramethyl benzidine peroxidase substrate and peroxidase solution B) was added and incubated for 15 min (IgA or IgM) or 30 min (IgG). Finally, 2 M H2SO4 was used to stop the 3,3′,5,5′-tetramethyl benzidine reaction. A microtiter plate reader (Wallac Victor-2 1420 Multilabel Counter, Perkin Elmer, Boston, MA) was used to measure the absorbance at 450 nm. To calculate the immunoglobulin (IgG, IgA, or IgM) concentration, a 4-parameter logistic curve fit was developed using the chicken reference serum absorbance.

**Leukocyte Isolation**

Peripheral blood was collected by decapitation and EDTA (EMD Chemicals Inc., Gibbstown, NJ) was used as an anticoagulant. Blood was pooled from 10 birds per treatment (n = 3 to 4 separate pools/gradient per treatment). The polymorphonuclear and mononuclear cell fractions were isolated as described elsewhere (Kogut et al., 1995). Briefly, the blood was mixed with a solution of 1% methylcellulose (Sigma-Aldrich), dissolved in RPMI-1640 media (Mediatech Inc., Herndon, VA) at a ratio of 1:1.5, and then centrifuged at 250 × g for 15 min. The supernatant was removed and resuspended in Hanks’ balanced salt solution without calcium and magnesium (Mediatech Inc.). The resulting suspension was layered over a 1.077/1.119 histopaque discontinuous gradient (Sigma-Aldrich) and centrifuged at 500 × g for 60 min. The top layer was collected for the mononuclear cell fraction and the interface of the 2 gradients was collected for heterophils. Cells types were then separated into different conical tubes and washed with 45 mL of RPMI-1640 by centrifugation at 500 × g for 30 min. After the RPMI-1640 wash, a complete solution of RPMI-1640 was then added to reconstitute the cell. The solution consisted of 5% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 4 mM L-glutamine, 10,000 U of penicillin/mL, 10 mg of streptomycin/mL (JR Scientific Inc., Woodland, CA), 1 mM sodium pyruvate (Mediatech Inc.), and 0.1 mM nonessential amino acids (MP Biomedicals LLC, Solon, OH). All agonists and substrates were suspended in this complete RPMI-1640. A working concentration of 4 × 10^6 heterophils/mL and 1 × 10^7 mononuclear cells/mL was kept in ice until use. Cell viability was determined by a commercially prepared trypan blue solution (Sigma-Aldrich).

**Oxidative Burst Assay**

Oxidative burst activity of heterophils and monocytes was measured using a Wallac fluorescent plate reader (Perkin Elmer) and an indicator of reactive oxygen species, 2,7-dichlorofluorescein (Sigma-Aldrich), as described previously (Xie et al., 2002). Cells (1 mL) were preincubated for 30 min with 125 µL of phorbol-12-myristate-13-acetate (20 µg/mL of cells, Calbiochem, La Jolla, CA) at 42°C in a water-jacketed 5% CO2 incubator. An equivalent volume of complete RPMI-1640 was added for the negative control treatments. Immediately after the preincubation period, 125 µL of 2,7-dichlorofluorescein (0.2 mg/mL) was added and samples were mixed and aliquoted into a clear 96-well flat-bottomed plate (24 wells per treatment). Oxidative burst was then measured at an excitation/emission wavelength at 485/530 nm.

**NO Assay**

Eight plasma samples per treatment, collected at 14 d PV and 14 d PI, were collected to determine total NO concentration. Blood samples were taken by venipuncture using blood collection tubes with EDTA as an anticoagulant (BD, Franklin Lakes, NJ) then centrifuged at 550 × g for 10 min at 4°C and the plasma was collected and stored at −80°C. The samples were thawed at 4°C to calculate the plasma NO2− + NO3− levels. A total NO assay kit (Assay Designs, Ann Arbor, MI; Nims et al., 1995) was used to determine both NO products in the sample. The kit allows the enzymatic conversion of
NO$_3^-$ to NO$_2^-$, followed by the colorimetric detection of NO$_2^-$ as a colored azo dye product of the Griess reaction, measured by absorbance at 585 nm using a Wallac microplate reader.

**Lesion Scoring**

At 6 d PV, and 7, 14, and 17 d PI, 10 birds per treatment were euthanized by cervical dislocation to score coccidial lesions in the intestines (upper and middle small intestine and ceca). After slaughter, the intestinal lesions were evaluated using a scale from 0 to 4 as described by Johnson and Reid (1970). Briefly, a lesion score of 0 indicates no gross lesions, whereas a lesion score of 4 indicates the maximum degree of pathogenicity. Lesions caused by *E. acervulina*, *E. maxima*, or *E. tenella* were analyzed together rather than individual species; therefore, the score range was adjusted per specific area of intestines.

**Statistical Analyses**

The data were analyzed as a 2-way ANOVA, with Arg and VE as the main effects, using the SigmaStat software (San Jose, CA). Because most of the interactions were significant, differences among means were separated by Tukey’s multiple comparison test using treatment means. Differences were considered statistically different when $P < 0.05$.

**RESULTS AND DISCUSSION**

Arginine is an essential amino acid in chickens because they do not possess a complete urea cycle (Tamir and Ratner, 1963); Arg supplementation above the NRC (1994) recommendations in poultry improves immune function (Potenza et al., 2001; Lee et al., 2002; Li et al., 2007). Vitamin E, a potent antioxidant compound in biological systems (Packer, 1991; Traber, 2007), has also been reported to have immunomodulatory effects (Erf et al., 1998; Meydani and Beharka, 1998; Boa-Ampounsem et al., 2000). Nevertheless, the effects of Arg or VE supplementation on the immune response and pathological progression after a coccidiosis challenge have been inconsistent (Colnago et al., 1984; Allen, 1999; Allen and Fettener, 2000, 2002).

We measured in vitro HOB and MOB as an indicator of heterophil and monocyte function (Figure 1) 7 d after coccidial infection. Heterophils, the counterpart of mammalian neutrophils (Powell, 1987), are the predominant granulated leukocytes in the acute inflammatory response in gallinaceous birds, possess a high phagocytic capability, and are able to produce antimicrobial activities (Harmon, 1998). Monocytes, which are immature macrophages, have phagocytic properties (Powell, 1987) and are capable of producing cytokines such as tumor necrosis factor-α, interleukin (IL)-1, and IL-6 to elicit the activation of neutrophils, monocytes, and macrophages to start bacterial and tumor cell destruction, raise adhesion molecule expression on the surface of neutrophils and endothelial cells, stimulate T- and B-lymphocyte proliferation, and initiate the production of other proinflammatory cytokines (Calder, 2007). At 7 d PI, the HOB was the lowest in birds fed VE40-NARG, but it was increased when combined with MARG and HARG levels; conversely, the HOB was higher in birds fed VE80 in combination with NARG and HARG but was depressed in birds fed VE80-MARG. The effects of Arg and VE on MOB were somehow similar to those on HOB, but the interaction between Arg and VE was stronger. For instance, birds fed high levels of VE in combination with NARG had higher MOB than birds fed VE80-MARG, whereas birds fed high levels of VE and HARG had intermediate values; on the other hand, birds fed normal levels of VE in combination with NARG or HARG had the lowest MOB, but birds fed VE40-MARG diets had significantly higher levels of MOB similar to that of birds fed VE80-NARG or VE80-HARG. Thus, our results show that high levels of VE alone are capable of improving both MOB and HOB when normal levels of Arg are fed but also show that the HOB and MOB can be significantly improved in diets with normal levels of VE with the addition of moderate amounts of Arg. Rose et al. (1984) reported a period of leukocytosis, mostly heterophils and lymphocytes, after a challenge with *E. maxima*; furthermore, local inflammatory responses are very important in early protection against coccidial infections, making the functionality of macrophages and heterophils important factors during coccidial infections. In the same way, recent reports highlighted the importance of inflammatory monocytes as a first line of defense in controlling intestinal pathogens (Dunay et al., 2008), showing that animals lacking inflammatory monocytes are unable to control parasitic infections. The same authors reported that these inflammatory monocytes upregulate the inducible NO synthase, produce IL-12, and secrete tumor necrosis factor-α in response to infection. Thus, by improving the functionality of heterophils and monocytes, high levels of VE or Arg, or their combination, may improve the ability of the birds to clear coccidial infections and to improve the acquired immunity.

The interaction effects between Arg and VE on MOB and HOB could be explained by the interactions between reactive oxygen species, NO, and VE. Activated monocytes (Cachia et al., 1998) and neutrophils (Naue-seef et al., 1991) convert molecular oxygen into a superoxide anion (O$_2^-$) by the enzyme NADPH oxidase. The O$_2^-$ formed by this enzyme is used for the production of a great variety of reactive oxidants, including oxidized halogens, free radicals, and singlet oxygen (Babior, 1999). In the luminal environment, the interaction of NO with reactive oxygen species leads to the catalytic conversion of several reactive nitrogen species, including NO$_2^-$, ONOO$^-$, N$_2$O$_3$, dinitrosyl iron complexes, nitrosothiols, and HNO. Reactive oxygen species and reactive nitrogen species synergize to exert...
highly toxic effects on intraphagosomal microorganisms (Flannagan et al., 2009). But on the other hand, VE is a potent antioxidant with antiinflammatory properties (Singh et al., 2005). Cachia et al. (1998) showed that α-tocopherol inhibits $\mathrm{O}_2^-$ production in human adherent monocytes by impairing the assembly of the NADPH oxidase. In another study, Fujii et al. (1997) found that NO suppresses the $\mathrm{O}_2^-$-generating activity of pig neutrophils by inhibiting the assembling process of NADPH oxidase.

Nitric oxide is an important mediator of innate and acquired immunity, and its levels in plasma increase after an *Eimeria* infection in a dose- and virulence-dependent manner (Allen, 1997a,b; Lillehoj and Li, 2004). In the present study, we did not find statistical differences in plasma NO among treatments 14 d after vaccination (data not shown). However, 14 d PI, the plasma NO levels were higher in birds fed the VE80-MARG or the VE80-NARG diet and lower in birds fed the VE40-NARG diet or the VE40-MARG diet, whereas birds fed the HARG diets had intermediate levels of NO regardless of the VE level (Figure 2). This strong interaction between Arg and VE on plasma levels of NO could explain the inconsistent results reported when only Arg or VE are supplemented in the diets of birds undergoing a coccidiosis infection. Allen (1999) reported that in chickens infected with *E. maxima*, *E. tenella*, or *E. acervulina*, Arg supplementation did not increase plasma levels of NO. In another study, chickens were experimentally inoculated with incremental doses of *E. acervulina* and fed 1.64% Arg; birds infected with the highest oocyst doses had the highest plasma NO levels (Allen and Fetterer, 2000). Regarding VE supplementation, the same authors reported that high levels of VE increased the plasma levels of NO in chickens facing a mild *E. tenella* infection, but VE supplementation did not affect NO plasma levels when the chickens were exposed to a severe *E. tenella* infection (Allen and Fetterer, 2002). Nevertheless, in all cases, higher levels of NO were associated with a stronger infection. Thus, our data suggest that birds vaccinated against coccidiosis and later challenged with a field mix of *Eimeria* species faced a less severe infection when fed diets VE40-MARG or VE80-NARG than when fed VE40-NARG or VE80-MARG diets.

Humoral-mediated immunity is stimulated by *Eimeria* infection (Abu Ali et al., 1976; Trees et al., 1985; Lillehoj and Trout, 1996), and it has been shown that antibody levels are related to the severity of the infection (Onaga et al., 1986) and level of exposure to the parasite (Gilbert et al., 1988). We measured total anti-
IgM and IgA levels in serum at 14 d PI. At 14 d PI, chickens fed the VE40-HARG diet had higher levels of IgG than birds fed the VE80-MARG or the VE80-HARG diet, whereas birds fed the VE40-MARG or the VE80-NARG diet had intermediate levels of IgG. However, at 14 d PI, birds fed the VE80-MARG diet had the highest level of IgG compared with the other groups, in which IgG levels were not significantly different. Overall, the levels of IgG were 3 to 4 times higher after infection than after vaccination, which agrees with the findings of Smith et al. (1993), which reported that IgG levels increased slightly after a primary *Eimeria* exposure but increased significantly after a challenge. Although it has been reported that antibody-mediated responses play minor roles in protection against coccidiosis (Lillehoj and Trout, 1996; Allen and Fetterer, 2002), Guzman et al. (2003) reported a relationship between high levels of IgG and low levels of oocyst shedding using a virulent vaccine. The IgG avian isotype is the predominant form in sera, produced after IgM in the primary antibody response and the main isotype generated in the secondary response; in fact, IgG is the major avian systemic antibody active in infections (Davison et al., 2008).

Chicken IgM is structurally and functionally homologous to its mammalian counterpart. This immunoglobulin is the predominant B-cell antigen receptor and during embryonic development is the first isotype to be expressed; IgG is the predominant isotype produced after initial exposure to a novel antigen (Davison et al., 2008). The IgM concentration at 14 d PV was higher in birds fed VE40-MARG or VE80-HARG than in birds fed VE80-NARG, whereas birds in the other treatments had intermediate and similar levels of IgM. At 14 d PI, birds fed VE40-HARG or VE80-HARG had higher levels of IgM compared with birds fed VE40-MARG or VE80-NARG. Specific IgM levels have been reported to increase after primary infection, with lower responses after secondary exposures (Mockett and Rose, 1986; Smith et al., 1993). Thus the levels of IgG and IgM were not consistently affected by dietary treatment after vaccination; however, after infection, birds fed high levels of VE in combination with medium levels of Arg had higher levels of IgG and IgM. It has been suggested that antibodies reduce the invasion of some species of *Eimeria* if parasites come into close contact with local antibodies before the parasite enters the host (Lillehoj and Trout, 1996). In this regard, birds fed high levels of VE and medium levels of Arg could be better prepared to respond to a field infection with *Eimeria* after vaccination.

The serum levels of IgA at 14 d PV had a different pattern of distribution, with birds fed VE40-NARG showing a higher level of IgA than birds fed either VE40-MARG or VE80-MARG; however, at 14 d PI, the serum levels of IgA were not different among birds fed different dietary treatments. The predominant form of antibody activity in bodily secretions is IgA. This secretory IgA provides a first line of defense against many pathogens (Davison et al., 2008). Thus, secretory IgA antibodies are more likely to be involved in resistance to parasites that infect the intestinal mucosa (Powell, 1987). However, Yun et al. (2000) pointed out that although identification of IgA in the bile or serum is a common method to assess *Eimeria* infection, it has no discriminating power due to the relatively low concentration of parasite-specific IgA in the presence of a high concentration of nonspecific antibody.

There were no noticeable gross lesions after the vaccination. Seven days PI, the lesions were mild in all areas of the intestine and not affected by dietary treatment (Table 2). At 14 d PI, the lesions in the upper small intestine tended to be lower in birds fed VE80 (P = 0.068) but were not affected by diet in the other sections of the intestine. At 17 d PI, birds fed the HARG diet had lower lesion scores in the upper small intestine than birds fed the MARG feed, but they were not different from those of the NARG-fed birds. Also, at 17 d PI, there was an interaction between Arg and VE on lesion scores of the middle small intestine: birds fed the VE40-HARG diet had a higher lesion score (1.2 ± 0.02) compared with birds fed the VE80-MARG or the VE80-NARG diets (0.0 and 0.3 ± 0.02, respectively).

### Table 1. Serum levels of immunoglobulins (ng/mL) in broiler chickens fed different levels of arginine and vitamin E<sup>1,2</sup>

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;3&lt;/sup&gt;</th>
<th>14 d&lt;sup&gt;4&lt;/sup&gt;</th>
<th>14 d PI&lt;sup&gt;5&lt;/sup&gt;</th>
<th>14 d&lt;sup&gt;4&lt;/sup&gt;</th>
<th>14 d PI&lt;sup&gt;5&lt;/sup&gt;</th>
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<th>14 d PI&lt;sup&gt;5&lt;/sup&gt;</th>
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<tr>
<td>VE40-NARG</td>
<td>151.0 ± 9.2&lt;sup&gt;b&lt;/sup&gt;d</td>
<td>940.3 ± 73.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.2 ± 3.5&lt;sup&gt;b&lt;/sup&gt;a</td>
<td>93.6 ± 6.4&lt;sup&gt;b&lt;/sup&gt;a</td>
<td>140.4 ± 25.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>367.1 ± 78.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>VE40-MARG</td>
<td>182.1 ± 9.6&lt;sup&gt;b&lt;/sup&gt;c</td>
<td>982.3 ± 90.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.5 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.6 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>827.0 ± 50.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.0 ± 7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.8 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.2 ± 16.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>357.2 ± 48.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>807.8 ± 54.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.7 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.4 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>245.8 ± 16.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1,509.0 ± 169.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>309.2 ± 33.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup>–<sup>d</sup>Means without a common superscript within the same column differ significantly (P < 0.05).

<sup>1</sup>The birds were vaccinated at d 1 and experimentally challenged at d 14 with a field mix of *Eimeria* (*Eimeria acervulina, Eimeria maxima, and Eimeria tenella*). Blood samples were taken at d 14 (before infection) and 14 d after infection [14 d postinfection (PI)].

<sup>2</sup>Values are the mean ± SEM of 8 observations.

<sup>3</sup>Birds were fed a corn-soybean meal-based diet meeting all of the NRC (1994) requirements with 40 or 80 IU of vitamin E/kg of feed (VE40 or VE80, respectively) and supplemented with l-arginine HCl at 0, 0.3, or 0.6% [normal level (NARG), medium level (MARG), or high level (HARG), respectively].
whereas birds in the other treatments had intermediate lesion score values (0.8, 0.7, and 0.9 ± 0.02 for the VE40-MARG, VE40-NARG, and VE80-NARG groups, respectively). Thus, the lower lesion score of birds fed the VE80-MARG diet was associated with the highest IgG levels at 14 d PI, indicating the positive effects of Arg and VE on the humoral immune response. However, the lesions were mild across all treatments because all chickens were vaccinated.

In summary, our results showed that high levels of VE alone are capable of improving both MOB and HOB when normal levels of Arg are fed, but they also showed that the HOB and MOB can be significantly improved in diets with normal levels of VE with the addition of moderate amounts of Arg. Also, plasma levels of NO suggested that birds vaccinated against coccidiosis and later challenged with a field mix of Eimeria species faced a less severe infection when fed NARG-VE80 or MARG-VE40 diets than when fed NARG-VE40 or MARG-VE80 diets. The levels of IgG and IgM were not consistently affected by dietary treatment after vaccination; however, after infection, birds fed high levels of VE in combination with medium levels of Arg had higher levels of IgG and IgM. These results suggest that birds fed higher levels of VE and Arg than those recommended by the NRC (1994) had a better immune response after an Eimeria challenge. However, further research is required to find out if the better immune response correlates with lower levels of oocyst shedding and better productive performance.

ACKNOWLEDGMENTS

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REFERENCES


Table 2. Coccidial infection-related lesion scores in the upper small intestine (U), medium small intestine (M), and lower intestine (ceca, L) of chickens fed different levels of vitamin E and arginine (2 × 3 factorial).1,2

<table>
<thead>
<tr>
<th>Item3</th>
<th>7 d PI</th>
<th></th>
<th></th>
<th>14 d PI</th>
<th></th>
<th></th>
<th>17 d PI</th>
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<tbody>
<tr>
<td></td>
<td>U</td>
<td>M</td>
<td>L</td>
<td>U</td>
<td>M</td>
<td>L</td>
<td>U</td>
<td>M</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.267</td>
<td>0.400</td>
<td>0.100</td>
<td>1.467</td>
<td>0.733</td>
<td>0.467</td>
<td>0.200</td>
<td>0.900</td>
</tr>
<tr>
<td>80</td>
<td>0.467</td>
<td>0.233</td>
<td>0.167</td>
<td>0.800</td>
<td>0.667</td>
<td>0.133</td>
<td>0.333</td>
<td>0.400</td>
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<tr>
<td>SEM</td>
<td>0.093</td>
<td>0.084</td>
<td>0.065</td>
<td>0.271</td>
<td>0.173</td>
<td>0.226</td>
<td>0.085</td>
<td>0.132</td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>NARG</td>
<td>0.300</td>
<td>0.200</td>
<td>0.150</td>
<td>1.500</td>
<td>0.600</td>
<td>0.100</td>
<td>0.290</td>
<td>0.734</td>
</tr>
<tr>
<td>MARG</td>
<td>0.550</td>
<td>0.350</td>
<td>0.150</td>
<td>0.900</td>
<td>0.700</td>
<td>0.500</td>
<td>0.500</td>
<td>0.400</td>
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<tr>
<td>HARG</td>
<td>0.250</td>
<td>0.400</td>
<td>0.100</td>
<td>1.000</td>
<td>0.800</td>
<td>0.300</td>
<td>0.100</td>
<td>0.750</td>
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<tr>
<td>SEM</td>
<td>0.114</td>
<td>0.103</td>
<td>0.079</td>
<td>0.332</td>
<td>0.212</td>
<td>0.277</td>
<td>0.104</td>
<td>0.162</td>
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<tr>
<td>P-value</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.151</td>
<td>0.168</td>
<td>0.470</td>
<td>0.696</td>
<td>0.894</td>
<td>0.319</td>
<td>0.176</td>
<td>0.008</td>
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<tr>
<td>Arginine</td>
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<td>0.368</td>
<td>0.576</td>
<td>0.290</td>
<td>0.734</td>
<td>0.676</td>
<td>0.025</td>
<td>0.082</td>
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<tr>
<td>Vitamin E × arginine</td>
<td>0.581</td>
<td>0.235</td>
<td>0.876</td>
<td>0.384</td>
<td>0.298</td>
<td>0.848</td>
<td>0.613</td>
<td>0.017</td>
</tr>
</tbody>
</table>

1Values without a common superscript within the same column and main effect differ significantly (P < 0.05).

2The intestinal lesions were evaluated using a scale from 0 to 4 as described by Johnson and Reid (1970). Briefly, a lesion score of 0 indicates no gross lesions, whereas a lesion score of 4 indicates the maximum degree of pathogenicity.

3Birds were fed a corn-soybean meal-based diet meeting all of the NRC (1994) requirements with 40 or 80 IU of vitamin E/kg of feed and supplemented with l-arginine HCl at 0, 0.3, or 0.6% (normal level (NARG), medium level (MARG), or high level (HARG), respectively).
ARGinine, VITamin E, AND COCCIDIOSIS

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