ABSTRACT
Research has shown that methionine+cysteine (M+C) requirements may be higher when chickens are infected with Eimeria app. In a 4 × 2 factorial design, broilers (11 to 21 D) were fed one of 4 corn–soybean meal-based diets containing either 0.6, 0.8, 0.9, or 1.0% standardized ileal digestible (SID) M+C; on day 14, broilers from each diet were gavaged with either phosphate-buffered saline (PBS) or a commercial coccidiosis vaccine (at 100× vaccine dose) which provided a mixture of live Eimeria acervulina, Eimeria maxima, and Eimeria tenella oocysts. Growth performance was recorded from day 11 to 21. Plasma and intestinal luminal samples were collected on days 14 and 21. Intestine lesion scores and fecal oocyst counts were conducted on day 21. Regardless of dietary SID M+C levels, compared to PBS gavaged broilers, the Eimeria-challenged broilers had (1) decreased (P < 0.05) body weight gain (BWG), feed intake (FI), and gain-to-feed ratio (G:F); (2) increased (P < 0.05) intestinal lesion scores and fecal oocyst counts; (3) increased (P < 0.05) plasma anti-Eimeria IgG, and intestinal luminal total IgA and anti-Eimeria IgA concentrations; and (4) increased (P < 0.05) levels of duodenum luminal gamma interferon (IFN-γ) and interleukin-10 (IL-10), as well as jejunum and cecum luminal IFN-γ concentrations. Regardless of Eimeria challenge, when compared to 0.6% SID M+C, broilers fed ≥0.8% SID M+C had (1) increased (P < 0.05) BWG, FI, and G:F and (2) increased (P < 0.05) levels of jejunum luminal total IgA. After Eimeria challenge, broilers fed 0.8% SID M+C had increased (P < 0.05) levels of jejunal luminal anti-Eimeria IgA compared to broilers fed diets containing 0.6 and 1.0% SID M+C. Collectively, in 11- to 21-D broilers, the growth suppression caused by Eimeria infection could not be mitigated by further increasing dietary M+C alone ≥0.8%. Further research should investigate interactions between dietary M+C and other nutrients for support of immune function and growth in pathogen-challenged broilers.

Key words: methionine, sulfur amino acid, broiler, Eimeria, intestinal IgA

INTRODUCTION
Coccidiosis, a parasitic intestinal disease caused by Eimeria infection, is one of the main challenges for the antibiotic-free poultry industry to overcome (Kadykalo et al., 2018). Previous work has shown that there is possibly an interaction between Eimeria infection and dietary sulfur amino acid levels in chickens. Murillo et al. (1976) reported that the growth performance was more seriously suppressed by Eimeria infection in Cobb broilers (1 to 28 D) fed a methionine+cysteine (M+C)-deficient diet (total M+C = 0.73%) compared to broilers fed a M+C-sufficient diet (total M+C = 0.83%). In a subsequent study, Southern and Baker (1982) observed that supplementation of 0.50% DL-methionine to a conventional corn–soybean meal based diet, to achieve a total M+C of 0.91%, partially alleviated the decrease in growth performance in Eimeria infected, cobalt-fed male chicks from 8 to 21 D of age. Lai et al. (2018) found that increasing dietary total methionine to at least 125% of the requirements (specified by the Ministry of Agricultural of the People’s Republic of China, 2004) could improve not only growth performance of male Partridge Shank broilers (from 22 to 42 D) in an Eimeria challenge, but also intestinal luminal levels of sIgA, and cecal tonsil mRNA expressions of tumor necrosis factor alpha (TNFα), interleukin-2 (IL-2), and gamma interferon (IFN-γ) in response to Eimeria challenge; however, the effects of increased dietary methionine were mostly observed for broilers reared with diets containing narasin (60 mg/kg),
rather than those conversely protected with a live anti-
coccidial vaccine administrated at day 3. These stud-
ies support a hypothesis that the sulfur amino acid requirement may be increased in *Eimeria*-challenged broilers compared to uninfected broilers. The require-
ments for M+C differ among the widespread nutrition
specifications. For 11- to 21-D broilers, the Ministry of
Agricultural of the People’s Republic of China (2004)
recommends a total M+C of 0.91%, while the major
broiler breeding companies recommend 0.80 and 0.87%
digestible M+C for Cobb 500 or Ross 308/708 broil-
ers, respectively. Amino acid providers may also of-
ffer recommendations; for example, AMINOChick 2.0
(Evonik Nutrition and Care GmbH, Hanau, Germany)
recommends standardized ileal digestible (SID) M+C of
0.83% for 11- to 21-D male broilers. Regardless of the
recommendations used to formulate diets, it remains to
be investigated whether or not the current sulfur amino
acid recommendations are adequate for broilers under-
going a health challenge, like an *Eimeria* infection.

The mode of action by which increased M+C has
reportedly protected broiler chickens from coccidiosis
related growth suppression is not defined. Two mecha-
nisms have been proposed: (1) *Eimeria* infection de-
creases the digestibility and/or absorption of M+C and
therefore increased supplementation is required (Ruff and Wilkins, 1984; Adedokun et al., 2012, 2016); and (2) M+C is additionally required to support im-
une response to pathogenic *Eimeria* spp. and there-
fore additional supplementation helps confer resis-
tance to *Eimeria*-infected chickens (Grimble and Grimble, 1998; Maroufyan et al., 2013; Wu et al., 2013). The
current study will focus on immune-related functions.
Previous research from our laboratory demonstrated
that plasma total IgG concentrations, in response to
sheep red blood cells, were linearly increased in 3-wk-
old broilers when dietary total M+C was increased from
0.72 to 0.97% (Tsiagbe et al., 1987b). In that study, the
dietary total M+C required for maximum broiler
growth was approximately 0.78%, but ≥0.97% total
M+C was required to maximize humoral immunity.
Similarly, Swain and Johri (2000) reported that broil-
ers fed 0.69% total methionine had a greater plasma
humoral immune response to Newcastle Disease Virus
when compared to broilers fed 0.37% total methionin-
e (dietary cysteine level was not given in this pa-
per). These findings were confirmed by Rama Rao
et al. (2003). Hence, we hypothesized that dietary sul-
fur amino acid supplementation is crucial for broilers
to develop humoral immunity against *Eimeria*. Secre-
tory IgA, the main class of antibody responsible for ar-
resting external pathogens at epithelial borders, plays a
critical role in maintaining intestinal health (Mantis
and Forbes, 2010; Chairatana and Nolan, 2017; Lycke
and Bemark, 2017). To date, very limited information
is available regarding the effects of dietary M+C on in-
testinal IgA production in *Eimeria*-challenged broilers.
In the current study, broilers (with or without *Eimeria*
challenge) were fed diets with different levels of dietary
SID M+C. Broiler growth performance, coccidiosis

| Item (%, unless noted) | Starter (1 to 10 D) | Grower (11 to 21 D) |
|------------------------|--------------------|--------------------|
| Corn                   | 57.26              | 60.06              |
| Soybean meal, 48% CP   | 35.00              | 31.36              |
| Soybean oil            | 3.14               | 4.09               |
| Dicalcium phosphate, 22% | 1.76              | 1.63               |
| Calcium carbonate      | 0.74               | 0.71               |
| Sodium chloride        | 0.37               | 0.37               |
| Choline chloride, 60%  | 0.10               | 0.10               |
| DL-Methionine, 99%     | 0.31               | 0.06               |
| L-Lysine sulfate, 54.6%| 0.24               | 0.17               |
| L-Threonine, 98.5%     | 0.08               | 0.05               |
| Premix1                | 1.00               | 1.00               |
| Sand                   | 0.40               |                    |
| In total               | 100.00             | 100.00             |

1Supplied per kilogram of diet: copper, 15 mg; iron, 40 mg; zinc, 18 mg; manganese, 100 mg; selenium, 0.35 mg; iodine, 1 mg; vitamin A, 10,000 IU; vitamin D3, 5,000 IU; vitamin E, 80 IU; vitamin K, 3 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 4 mg; vitamin B12, 0.02 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; biotin, 0.15 mg; folic acid, 2 mg.  
2AMEn, nitrogen-corrected apparent metabolizable energy; SID, stan-
dardized ileal digestible. SID levels were calculated based on poultry specific digestibility coefficients provided by Evonik Nutrition and Care
GmbH (Hanau, Germany).

MATERIALS AND METHODS
All animal protocols conducted were approved by the
College of Agricultural and Life Sciences Animal Care
and Use Committee at the University of Wisconsin-
Madison.

Broilers and Diets
One-day-old male broilers (n = 720; Welp Hatchery,
Bancroft, IA) were randomly allotted to 80 battery
 cages with 9 broilers per cage. Broilers were fed with the
same nutritionally adequate corn–soybean meal-based
starter diet containing 0.88% SID M+C from 1 to 10 D
(Table 1). On day 11, the 80 cages were equally assigned
to one of 4 grower diets formulated to meet SID require-
ments (AMINOChick 2.0) except for M+C. The 4 diets
contained 0.6, 0.8, 0.9, and 1.0% SID M+C, which was
accomplished by adjusting the additions of subliminal
pathology, and immune response were evaluated. We
hypothesize that the optimal amino acid levels and ra-
tios may shift during the immune response to a chal-
lenge as requirements for functional amino acids, like
M+C, increase to support immune function in addition
to growth. More specifically, we hypothesize that the
current recommended SID M+C levels are not adequate
for *Eimeria*-infected broilers, and increased dietary sup-
plementation of methionine may improve *Eimeria* spp.
resistance and growth performance during a challenge.

| Item (%, unless noted) | Starter (1 to 10 D) | Grower (11 to 21 D) |
|------------------------|--------------------|--------------------|
| Corn                   | 57.26              | 60.06              |
| Soybean meal, 48% CP   | 35.00              | 31.36              |
| Soybean oil            | 3.14               | 4.09               |
| Dicalcium phosphate, 22% | 1.76              | 1.63               |
| Calcium carbonate      | 0.74               | 0.71               |
| Sodium chloride        | 0.37               | 0.37               |
| Choline chloride, 60%  | 0.10               | 0.10               |
| DL-Methionine, 99%     | 0.31               | 0.06               |
| L-Lysine sulfate, 54.6%| 0.24               | 0.17               |
| L-Threonine, 98.5%     | 0.08               | 0.05               |
| Premix1                | 1.00               | 1.00               |
| Sand                   | 0.40               |                    |
| In total               | 100.00             | 100.00             |
| Nutritional composition (calculated)2 | 3,008 | 3,086 |
| Crude protein, %       | 21.74              | 20.00              |
| SID Methionine, %      | 0.59               | 0.33               |
| SID Cysteine, %        | 0.29               | 0.27               |
| SID Methionine + Cysteine, % | 0.88 | 0.60 |
| SID Lysine, %          | 1.18               | 1.05               |
| SID Threonine, %       | 0.77               | 0.69               |
Table 2. Analyzed nutrient levels of experimental diets (as-is basis).

| Item (%)  | Calculated | Analyzed | Calculated | Analyzed  |
|-----------|------------|----------|------------|-----------|
|           | 0.6% SID M+C | 0.8% SID M+C | 0.9% SID M+C | 1.0% SID M+C |
| Crude protein | 21.74 | 21.27 | 20.00 | 18.80 | 19.54 | 20.16 | 20.16 | 18.87 |
| Methionine  | 0.74 | 0.52 | 0.35/0.55/0.65/0.75 | 0.33 | 0.50 | 0.61 | 0.61 | 0.72 |
| Cysteine   | 0.34 | 0.34 | 0.33 | 0.31 | 0.32 | 0.32 | 0.32 | 0.30 |
| Methionine + Cysteine | 0.96 | 0.86 | 0.68/0.88/0.98/1.08 | 0.64 | 0.81 | 0.93 | 0.93 | 1.02 |
| Lysine     | 1.29 | 1.26 | 1.15 | 1.11 | 1.14 | 1.14 | 1.14 | 1.07 |
| Threonine  | 0.89 | 0.85 | 0.80 | 0.75 | 0.77 | 0.77 | 0.77 | 0.75 |
| Arginine   | 1.44 | 1.38 | 1.32 | 1.24 | 1.27 | 1.26 | 1.26 | 1.19 |
| Isoleucine | 0.91 | 0.90 | 0.84 | 0.81 | 0.83 | 0.82 | 0.82 | 0.76 |
| Leucine    | 1.82 | 1.77 | 1.72 | 1.61 | 1.63 | 1.63 | 1.63 | 1.54 |
| Valine     | 1.00 | 0.98 | 0.93 | 0.89 | 0.91 | 0.90 | 0.90 | 0.86 |
| Histidine  | 0.57 | 0.55 | 0.53 | 0.49 | 0.51 | 0.50 | 0.50 | 0.48 |
| Phenylalanine | 1.07 | 1.08 | 0.99 | 0.98 | 0.99 | 0.99 | 0.99 | 0.92 |
| Glycine    | 0.88 | 0.85 | 0.82 | 0.77 | 0.78 | 0.78 | 0.78 | 0.73 |
| Serine     | 1.06 | 1.03 | 0.98 | 0.94 | 0.95 | 0.95 | 0.95 | 0.91 |
| Proline    | 1.21 | 1.27 | 1.17 | 1.17 | 1.13 | 1.14 | 1.14 | 1.05 |
| Alanine    | 1.06 | 1.01 | 1.00 | 0.93 | 0.94 | 0.94 | 0.94 | 0.92 |
| Aspartate aminotransferase | 2.22 | 2.16 | 2.04 | 1.94 | 1.99 | 1.98 | 1.98 | 1.88 |
| Glutamic acid | 3.86 | 3.68 | 3.58 | 3.34 | 3.40 | 3.38 | 3.38 | 3.21 |

1Amino acid content of treatment diets were analysed by ion-exchange chromatography (AA analyser LC 3000, Biotronic, Maintal, Germany). SID M+C, standardized ileal digestible methionine + cysteine.

DL-methionine (MetAMINO 99%, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) at levels of 0, 0.2, 0.3, and 0.4%, respectively, in place of sand (River Run Products Corp., Custer, WI) as an inert ingredient (Table 1). The composition and calculated nutrient levels of the experimental diets are listed in Table 1. Amino acid content of treatment diets were analyzed by ion-exchange chromatography (AA analyser LC 3000, Biotronic, Maintal, Germany). The analyzed nutrient levels of the experimental diets are listed in Table 2. On day 14, broilers fed each grower diet group were orally gavaged with either phosphate-buffered saline (PBS) or a commercial coccidiosis vaccine dose; Advent, Lincoln, NE. The vaccine provided a mixture of live Eimeria acervulina, Eimeria maxima, and Eimeria tenella oocysts. Body weight gain (BWG), gain-to-feed ratio (G:F), and feed intake (FI) of the broilers were recorded/calculated at the following intervals: from 11 to 14 D, from 15 to 21 D, and from 11 to 21 D of age. On days 14 and 21, 1 bird per cage was randomly selected and euthanized for collection of plasma and intestinal (duodenum, jejunum, ileum, and cecum) luminal samples. On day 21, the intestine of sampled birds were subjected to a lesion scoring system described in Johnson and Reid (1970), ranging from a score of 0 (no gross lesion) to 4 (serve gross lesions). On day 21, the intestine of sampled birds were subjected to a lesion scoring system described in Johnson and Reid (1970), ranging from a score of 0 (no gross lesion) to 4 (serve gross lesions). On day 21, the intestine of sampled birds were subjected to a lesion scoring system described in Johnson and Reid (1970), ranging from a score of 0 (no gross lesion) to 4 (serve gross lesions). On day 21, the intestine of sampled birds were subjected to a lesion scoring system described in Johnson and Reid (1970), ranging from a score of 0 (no gross lesion) to 4 (serve gross lesions). On day 21, the intestine of sampled birds were subjected to a lesion scoring system described in Johnson and Reid (1970), ranging from a score of 0 (no gross lesion) to 4 (serve gross lesions).

Anti-Eimeria Antibody Enzyme-Linked Immunosorbent Assay (ELISA)

To create the plate coating antigen for ELISA, the Advent coccidiosis vaccine (10 mL; 10,000× vaccine dose) was diluted (1:2) with PBS and then centrifuged at 3,000× g for 10 min. The supernatant was discarded and the pellet was washed with 10 mL PBS (add PBS, suspend, centrifuge at 3,000× g for 10 min, and discard supernatant). The pellet was then diluted in 2 mL PBS and agitated with 4 mL glass beads on a vortex, until the pellet was thoroughly dispersed. The breakdown of oocysts was verified using a microscope (10×). The supernatant (broken oocysts) was pipetted off and the glass beads were washed (add PBS, vortex, and pipet supernatant off) 3 times using 8, 8, and 7 mL PBS, respectively. The supernatant (2+8+8+7 = 25 mL) was subjected to 2 freeze-thaw cycles to further ensure oocyst disruption. After that, the supernatant, which served as plate-coating Eimeria antigen in our anti-Eimeria antibody ELISA, was stored in 1 mL aliquots at −20°C until required for use. Anti-Eimeria antibody positive broiler plasma and intestinal lumen contents, obtained from another research project, were used as in-lab standards. Seven-point standard curves were obtained by using the 2-fold serial dilutions from the standard plasma (diluted in a protein-free blocking buffer from Pierce, Thermo Scientific, Rockford, IL; from 1:25 to 1:1600) and the standard intestinal lumen content (diluted in 1% milk powder; from 1:2 to 1:128). For plasma anti-Eimeria antibody ELISA, samples were diluted to 1:200, and the secondary detection antibody (goat anti-chicken IgG [IgY]–horseradish peroxidase [HRP] conjugated; Bethyl Laboratories, Inc., Montgomery, TX) was diluted to 1:4000, using the aforementioned protein-free blocking buffer. For intestinal lumen content anti-Eimeria antibody ELISA, duodenum, jejunum, ileum, and cecum luminal samples were diluted to 8, 3, 5, and 7 mg/mL, respectively, and the second antibody (goat anti-chicken IgA–HRP conjugated; Bethyl Laboratories, Inc., Montgomery, TX)
was diluted to 1:4000, using 1% milk powder. Protein concentrations of intestinal luminal samples were determined using Pierce BCA Protein Assay Kit (Thermo Scientific).

At the time of ELISA, the *Eimeria* antigen (1 mL) was diluted in 10 mL of carbonate coating buffer (pH = 9.6) and coated (100 μL/well, 4°C, overnight) to a Costar EIA/RIA 96-well plate (Corning Inc., Corning, NY). The plate was washed twice with PBS Tween 20 (Thermo Fisher Scientific, Rockford, IL) and then blocked (room temperature, ~2 h) with the aforementioned protein-free blocking buffer (200 μL/well). The standards, blanks, and samples were then added into the wells at 100 μL/well. The plates were incubated (1 h) on an orbital shaker at room temperature. The plate was then washed 4 times, the secondary detection antibody was diluted, applied to each well (100 μL/well), and incubated (1 h) on a shaker at room temperature. Plates were then washed 6 times, and a Pierce 1-Step Ultra TMB-ELISA Substrate Solution (Thermo Scientific) was applied at 100 μL/well. The colorimetric reaction was stopped by addition of 50 μL/well of a 0.5 M H₂SO₄ solution. The plates were read at 450 nm wavelength using an EL800 plate reader (BioTek, Winooski, VT). The log₂ anti-*Eimeria* antibody titer was calculated by comparing samples with the in-lab standard. The antibody titer was defined as the highest dilution of sample with an optical density equal to the standard plasma diluted 1:1600 (cutoff value, two times of the background) or the standard intestinal lumen content diluted 1:128 (cutoff value, two times of the background).

**Intestinal Luminal Total IgA ELISA**

Intestinal luminal concentrations of total IgA were determined using a commercial Chicken IgA ELISA Quantitation Set (E30-103) according to the manufacturer’s specifications (Bethyl Laboratories, Inc., Montgomery, TX). Briefly, affinity purified goat anti-chicken IgA antibody (A30-103A-19) was coated to the 96-well plates. The plates were blocked after washing. The standards (chicken reference serum RS10-102-3), blanks, and samples (duodenum, jejunum, ileum, and cecum luminal samples were diluted to 1:1000, 1:1500, 1:1000, and 1:500, respectively, prior to analysis) were then added into the wells. After incubation, the plates were washed and streptavidin-HRP solution (Lot # 10A8/1) was added. After incubation, the plates were washed and streptavidin-HRP solution (Lot # 10A8/1) was added. The colorimetric reaction was induced and then stopped by TMB and 0.5 M H₂SO₄, respectively. The plates were read at a wavelength of 450 nm. Data were expressed as ng/mL (plasma) and pg/mg protein (intestinal luminal).

**IL-10 ELISA**

Details regarding the IL-10 capture ELISA had been carefully described in our previous publication (Arendt et al., 2016). In the current study, the plasma samples were diluted to 1:500 prior to analysis and the results were recorded as μg/mL. The duodenum, jejunum, ileum, and cecum luminal samples were diluted to 1:5, 1:4, 1:4, and 1:5, respectively, prior to analysis, and the results were recorded as ng/mg protein.

**IFN-γ ELISA**

Plasma and intestinal luminal levels of IFN-γ were determined using a Chicken IFN-γ Cytoset ELISA kit (Lot # 102803) according to the manufacturer’s specifications (Invitrogen Corporation, Camarillo, CA). Briefly, anti-chicken IFN-γ antibody (Lot # 10F16/1) was coated to the 96-well plates. The plates were blocked after washing. The standards (recombinant chicken IFN-γ, Lot # 7C4/1), blanks, and samples (the plasma samples were diluted to 1:200, and the intestinal luminal samples were not diluted, prior to analysis) were then added into the wells. After washing, the detection antibody (anti-chicken IFN-γ biotin, Lot # 10F18/1) was added. After incubation, the plates were washed and streptavidin-HRP solution (Lot # 10A8/1) was added. The colorimetric reaction was induced and then stopped by TMB and 0.5 M H₂SO₄, respectively. The plates were read at a wavelength of 450 nm. Data were expressed as ng/mL (plasma) and pg/mg protein (intestinal luminal).

**Statistics**

Data were analyzed using General Linear Model (GLM) procedures (SPSS 23, IBM Corp., Chicago, IL) to detect differences among treatments based on a 4 x 2 factorial arrangement. The main effects were (1) dietary SID M+C levels (0.6, 0.8, 0.9, and 1.0%) and (2) *Eimeria* challenge (with or without). Post hoc analysis (Duncan’s test) was conducted to detect differences among all experimental treatments or differences within each main effect. Intestinal lesion scores were transformed to square root of n+1 prior to analysis. Statistical significance was set at P < 0.05.

**RESULTS**

**Growth Performance**

Pooled across *Eimeria*-challenged treatments, which were not yet imposed, broilers fed 0.6% SID M+C over the 11 to 14 D interval had decreased (P < 0.05) BWG compared to broilers fed 0.8 and 1.0% SID M+C, and had decreased (P < 0.05) FI compared to broilers fed 0.8% SID M+C (Table 3). During the intervals of 15 to 21 D and 11 to 21 D, broilers fed 0.6% SID M+C (pooled across main effects of *Eimeria* challenge) had decreased (P < 0.05) BWG and G:F ratio compared to broilers fed with 0.8, 0.9, and 1.0% SID M+C. From 15 to 21 D, BWG, G:F ratio, and FI were decreased (P < 0.05) by 18, 12, and 8% respectively, in...
Table 3. Growth performance of broilers fed varying levels of sulfur amino acids in control conditions or challenged with *Eimeria* spp.1

| Challenge | Day 11–21 | Day 11–14 | Day 15–21 | Day 11–21 | Day 11–14 | Day 15–21 | Day 11–21 | Day 11–14 | Day 15–21 | Day 11–21 |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| BWG (g)   | 0.6       | 127       | 255       | 382       | 0.64      | 0.55      | 0.58      | 198       | 457       | 651       |
| G:F (g:g) | 0.8       | 155       | 330       | 485       | 0.68      | 0.67      | 0.67      | 231       | 498       | 729       |
| FI (g)    | 0.9       | 139       | 336       | 474       | 0.68      | 0.70      | 0.69      | 203       | 483       | 687       |
|           | 1.0       | 154       | 341       | 495       | 0.69      | 0.67      | 0.68      | 222       | 509       | 730       |
|          | 0.6       | 124       | 218       | 342       | 0.66      | 0.46      | 0.53      | 196       | 421       | 621       |
| Eimeria   | 0.8       | 148       | 277       | 425       | 0.70      | 0.60      | 0.63      | 211       | 466       | 673       |
|           | 0.9       | 137       | 271       | 407       | 0.69      | 0.60      | 0.63      | 203       | 452       | 652       |
|          | 1.0       | 139       | 269       | 408       | 0.66      | 0.61      | 0.63      | 209       | 445       | 652       |
| PBS       | 0.6       | 137       | 315       | 459       | 0.68      | 0.65      | 0.65      | 205       | 487       | 699       |
| Eimeria   | 0.8       | 144       | 315       | 459       | 0.68      | 0.64      | 0.64      | 213       | 477       | 691       |

SEM 3 9 10 0.01 0.02 0.01 3 10 12

**Main effect**

| SID M+C (%) | 0.6      | 125b     | 237b     | 362b     | 0.65     | 0.50b    | 0.55b    | 197b     | 439     | 636     |
|-------------|----------|----------|----------|----------|----------|----------|----------|----------|---------|---------|
|             | 0.8      | 152a     | 303a     | 455a     | 0.69     | 0.63a    | 0.65a    | 221a     | 482     | 701     |
|             | 0.9      | 138ab    | 303a     | 441a     | 0.68     | 0.65a    | 0.66a    | 203ab    | 468     | 669     |
|             | 1.0      | 147a     | 305a     | 452a     | 0.68     | 0.64a    | 0.65a    | 216ab    | 477     | 691     |
| PBS         | 0.6      | 137      | 315      | 459      | 0.68     | 0.65a    | 0.65a    | 205      | 487     | 699ab   |
| Eimeria     | 0.8      | 144      | 259b     | 396b     | 0.67     | 0.57b    | 0.60b    | 214      | 446     | 650b    |

**SEM**

3 9 10 0.01 0.02 0.01 3 10 12

**P-value**

| SID M+C | 0.001     | 0.004     | 0.001     | 0.610     | 0.002     | <0.001    | 0.044     | 0.408     | 0.196    |
|---------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| Challenge | 0.143     | <0.001    | 0.001     | 0.871     | 0.006     | 0.002     | 0.178     | 0.040     | 0.031    |
| Interaction | 0.760     | 0.855     | 0.819     | 0.852     | 0.983     | 0.940     | 0.608     | 0.929     | 0.867    |

**Means with different superscripts within a column were significantly different (P < 0.05).**

**SID M+C, standardized ileal digestible methionine + cysteine; BWG, body weight gain; G:F, gain to feed ratio; FI, feed intake; PBS, phosphate-buffered saline.**

**Challenge on day 14, broilers were orally gavaged with either PBS or the Advent Coccidiosis Vaccine (Lincoln, NE; 100x vaccine dose; consisting of a blend of live *Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella* oocysts).**

**Table 4. Intestinal lesion score and fecal oocyst counts of 21-day-old broilers exposed to an *Eimeria* spp. challenge.**

| Challenge | SID M+C (%) | Duodenum | Jejunum | Cecum | Fecal oocyst (No./g) |
|-----------|-------------|----------|---------|-------|---------------------|
| Eimeria   | 0.6         | 1.40     | 1.20    | 1.60  | 632,267             |
| Eimeria   | 0.8         | 1.20     | 0.60    | 1.70  | 618,933             |
| Eimeria   | 0.9         | 1.20     | 0.80    | 1.70  | 342,933             |
| Eimeria   | 1.0         | 1.40     | 1.00    | 1.40  | 561,333             |
| SEM       | 0.094       | 0.209    | 0.951   | 0.256 |

**SEM 0.12 0.12 0.23 0.002 0.040 0.196 0.381 0.040 0.031 0.050**

**P-value**

**Eimeria-challenged broilers.** From 11 to 21 D, BWG, G:F ratio, and FI were decreased (P < 0.05) by 14, 8, and 7%, respectively, in *Eimeria*-challenged broilers. No interactions (P > 0.05) between the main effects of SID M+C levels and *Eimeria* challenge were detected for any of the growth performance traits (Table 3).

**Intestinal Lesion Score, Fecal Oocyst Counts, and Plasma Anti-*Eimeria* IgG Titer**

Intestine lesion scores and excreta *Eimeria* oocysts were detected in *Eimeria*-challenged broilers, but not PBS gavaged controls (Table 4). On day 21, *Eimeria*-challenged broilers had increased (P < 0.05) plasma anti-*Eimeria* IgG concentrations compared to PBS gavaged broilers (Table 5). Dietary SID M+C levels had no effect (P > 0.05) on intestinal lesion score (Table 4), fecal oocyst counts (Table 4), and plasma anti-*Eimeria* IgG concentrations (Table 5). No statistical differences (P > 0.05) were attributed to the interactions of dietary SID M+C × *Eimeria* challenge treatments, although the greatest anti-*Eimeria* IgG concentrations were detected in *Eimeria*-challenged broilers fed 0.9 and 1.0% SID M+C.


Table 5. Plasma anti-\textit{Eimeria} IgG titer of broilers fed varying levels of sulfur amino acids in control conditions or challenged with \textit{Eimeria} spp.\textsuperscript{1}

| Challenge\textsuperscript{2} | SID M+C (%)\textsuperscript{3} | Day 11–21 | Day 14 | Day 21 |
|-------------------------------|-------------------------------|-----------|--------|--------|
| PBS                           | 0.6                           | 10.5      | 9.7    |        |
|                               | 0.8                           | 10.5      | 9.4    |        |
|                               | 0.9                           | 10.5      | 9.6    |        |
|                               | 1.0                           | 10.4      | 9.8    |        |
| \textit{Eimeria}              | 0.6                           | 10.5      | 10.5   |        |
|                               | 0.8                           | 10.3      | 10.6   |        |
|                               | 0.9                           | 10.5      | 11.0   |        |
|                               | 1.0                           | 10.4      | 11.1   |        |
| SEM                           |                               | 0.1       | 0.1    |        |
| Main effect                   |                               |           |        |        |
|                               | 0.6                           | 10.5      | 10.4   |        |
|                               | 0.8                           | 10.4      | 10.3   |        |
|                               | 0.9                           | 10.5      | 10.5   |        |
|                               | 1.0                           | 10.4      | 10.7   |        |
| PBS                           | 10.5                          |           | 9.8\textsuperscript{a} |        |
| \textit{Eimeria}              | 10.4                          |           | 11.0\textsuperscript{a} |        |

\textsuperscript{a,b}Means with different superscripts within a column were significantly different (\emph{P}< 0.05).

\textsuperscript{1}Titers were defined as Log 2 of the highest dilution of sample with an optical density equal to the standard plasma diluted 1:1600 (cutoff, two times of the background). The average maternally derived anti-\textit{Eimeria} IgG titer was 14.3 in the plasma of 1-day-old broilers (data not shown).

\textsuperscript{2}On day 14, broilers were orally gavaged with either PBS or the Advent Coccidiosis Vaccine (Lincoln, NE; 100× vaccine dose; consisting of a blend of live \textit{Eimeria acervulina}, \textit{Eimeria maxima}, and \textit{Eimeria tenella} oocysts).

\textsuperscript{3}SID M+C, standardized ileal digestible methionine + cysteine; PBS, phosphate-buffered saline.

**Plasma and Intestinal Luminal Levels of IL-10 and IFN-γ**

Differences in plasma IL-10 and IFN-γ concentrations were not detected among dietary SID M+C or \textit{Eimeria} challenge groups on day 21 (Table 8). On day 21, \textit{Eimeria} challenge increased (\emph{P}< 0.05) duodenum luminal concentrations of IL-10 and IFN-γ, and increased (\emph{P}< 0.05) jejunum and cecum luminal levels IFN-γ (Table 9). Dietary SID M+C levels or the SID M+C × \textit{Eimeria} interaction had no effect (\emph{P}> 0.05) on intestinal luminal IL-10 and IFN-γ concentrations.

**DISCUSSION**

The current study was conducted in order to investigate whether increasing levels of dietary sulfur amino acids may offer improved immune response, growth performance of broilers exposed to \textit{Eimeria} spp. parasites. Methionine is not only the first limiting amino acid for broiler growth but has also been shown to play vital roles in immune function. Methionine is a precursor of polyamines, required for immune cell proliferation, induction/regulation of inflammation and pathogen recognition (Minois et al., 2011; Correa-Fiz et al., 2012). Sulfur amino acids are also required for the production of glutathione which has been shown to enhance immune responses to intracellular parasites, improve proliferation or adaptive immune cells and control oxidants produced during inflammation (Dröge et al., 1998; Morris et al., 2013; Hughes et al., 2017). Additionally, intestinal infections like those with \textit{Eimeria} spp. pathogens have been shown to reduce the digestibility of M+C (Adedokun et al., 2016) as well as other functional amino acids like threonine, valine, isoleucine, arginine, and lysine (Persia et al., 2006). Specifically, malabsorption of L-methionine was observed during \textit{Eimeria} spp. challenge (Ruff and Wilkins, 1980). As the protective immune response to \textit{Eimeria} spp. infections involves many of these immune processes, it was hypothesized that coccidiosis increases the broilers’ M+C requirement in order to maintain growth performance while additionally providing essential substrates for immune function.

Regardless of \textit{Eimeria} challenge, broilers fed 0.8, 0.9 and 1.0% SID M+C exhibited greater growth than those fed 0.6% SID M+C, while no difference was observed among broilers fed diets with ≥0.80% SID M+C. Under the current experimental conditions (from 11 to 21 D), a corn–soybean meal-based diet containing ≥0.80% SID M+C was adequate for maximum growth performance for both normal broilers and \textit{Eimeria}-challenged broilers. Similarly, a dietary total M+C level of ≥0.87% was sufficient to maintain the antioxidant status (Wang et al., 2018) and meat quality (Wen et al., 2017) of 1- to 21-D broilers. These responses to dietary SID M+C are consistent with the current industry feeding practices for dietary M+C supplementation and support the reliability of M+C.

**Intestinal Luminal Anti-\textit{Eimeria} IgA and Total IgA Concentrations**

On day 14 (before \textit{Eimeria} challenge), no difference (\emph{P}> 0.05) was detected in intestinal luminal anti-\textit{Eimeria} IgA concentrations (Table 6) or in total IgA concentrations (Table 7) among treatments. \textit{Eimeria} challenge increased (\emph{P}< 0.05) duodenum, jejunum, ileum, and cecum luminal concentrations of anti-\textit{Eimeria} IgA (Table 6), and increased (\emph{P}< 0.05) duodenum, jejunum, and ileum luminal concentrations of total IgA (Table 7), on day 21. Broilers fed 0.6% SID M+C tended to have decreased (\emph{P}= 0.067) concentrations of jejunum luminal total IgA on day 21 compared to broilers fed 0.8, 0.9 and 1.0% SID M+C, regardless of \textit{Eimeria} challenge. An interaction tendency (\emph{P}= 0.067) was detected on day 21 jejunum luminal anti-\textit{Eimeria} IgA concentrations (Table 6). Briefly, dietary SID M+C levels had no effect on day 21 jejunum luminal anti-\textit{Eimeria} IgA concentrations in PBS gavaged broilers; however, \textit{Eimeria}-challenged broilers fed 0.8% SID M+C had increased jejunum luminal anti-\textit{Eimeria} IgA concentrations at day 21, compared to broilers fed diets containing 0.6 and 1.0% SID M+C.
specifications from academic groups (e.g., Ministry of Agricultural of the People's Republic of China, 2004), the major broiler breeding companies (e.g., Cobb 500, Ross 308, and Arbor Acres), and amino acid providers (e.g., EVONIK Nutrition and Care GmbH). An *Eimeria* challenge suppressed the growth of broilers regardless of dietary SID M+C levels. After challenge, the day 15 to 21 BWG was decreased by 15, 16, 19, and 21% in broilers fed 0.6, 0.8, 0.9, and 1.0%, respectively. In *Eimeria*-challenged broilers, feeding diets formulated with ≥0.8% SID M+C significantly increased growth performance when compared to 0.6% SID M+C. This response reflects a simple SID M+C deficiency at 0.6%, but does not infer that the additional supplemental SID M+C functioned against *Eimeria*. In addition, linear and quadratic effects of dietary SID M+C levels on growth performance have been tested using unequal coefficient calculations according to Carmer and Seif (1963). However, the results show a clear “lack of fit”, in both the *Eimeria*-challenged and non-challenged groups, if we force a linear or even a quadratic curve fit (data not shown). Hence, we report that *Eimeria*-induced growth suppression was not mitigated by simply increasing dietary SID M+C levels.

The current *Eimeria* challenge model was an effective immune challenge as confirmed by intestine lesion scores and fecal oocyst count. No intestinal lesions were observed in the ileum of *Eimeria*-challenged broilers since *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* are specific for duodenum, jejunum, and cecum, respectively. As expected, after *Eimeria* challenge, a significant humoral immune response was observed in broiler plasma and intestinal luminal samples, regardless of dietary SID M+C levels. Interestingly, broilers fed ≥0.8% SID M+C, irrespective of *Eimeria* infection, had increased levels of jejunum luminal total IgA concentrations when compared to broilers fed 0.6% SID M+C. Similar findings were reported in Rama Rao et al. (2003), where antibody response to sheep red blood cells and resistance to *Escherichia coli* were enhanced in broilers by increasing dietary total M+C levels from 0.72 to 0.88%. These results are supported by previous findings (Tsiagbe et al., 1987b) and are in good agreement with the general understanding that the nutritional status of sulfur amino acid is important to humoral immunity (Bouyeh, 2012; Jahnian and Khalifeh-Gholi, 2018; Lai et al., 2018). Of particular note, within the *Eimeria*-challenged groups, broilers fed 0.8% SID M+C had higher concentrations of jejunum luminal anti-*Eimeria* IgA when compared to broilers fed 0.6 and 1.0% SID M+C. Apparently, 0.6% SID M+C was not adequate for broilers to maximize intestinal anti-*Eimeria* IgA concentrations. However, further studies will need to confirm whether 1.0% SID M+C is too high and potentially cause adverse effects on intestinal anti-*Eimeria* IgA production in 11- to 21-D broilers. We previously reported that >0.97% dietary M+C depressed antibody responses (to sheep red blood cells) in 14- to 24-D broilers (Tsiagbe et al., 1987a). Bhargava et al. (1970) reported that the

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**Table 6. Intestinal luminal anti-*Eimeria* IgA titer of broilers fed varying levels of sulfur amino acids in control conditions or challenged with *Eimeria* spp.**

| Challenge<sup>2</sup> | SID M+C (%)<sup>3</sup> | Day 14 | | Day 21 | | |
|------------------------|------------------------|--------|--------|--------|--------|--------|
|                        | Duodenum | Jejunum | Ileum | Cecum<sup>4</sup> | Duodenum | Jejunum | Ileum | Cecum<sup>4</sup> |
| **PBS**                |          |         |       |         |          |         |       |         |
| 0.6                    | 2.50     | 3.34    | 1.67  | –       | 2.80     | 4.23<sup>a</sup> | 2.80  | 1.31  |
| 0.8                    | 2.88     | 3.44    | 2.26  | –       | 2.79     | 4.12<sup>a</sup> | 2.83  | 1.42  |
| 0.9                    | 2.56     | 3.42    | 1.99  | –       | 3.02     | 4.08<sup>b</sup> | 2.98  | 1.31  |
| 1.0                    | 2.91     | 3.61    | 2.11  | –       | 3.36     | 4.16<sup>c</sup> | 2.50  | 1.33  |
| **Eimeria**            |          |         |       |         |          |         |       |         |
| 0.6                    | 2.75     | 3.99    | 2.36  | –       | 4.65     | 5.27<sup>c</sup> | 4.17  | 1.99  |
| 0.8                    | 2.89     | 3.51    | 2.04  | –       | 5.07     | 6.51<sup>b</sup> | 4.92  | 1.71  |
| 0.9                    | 2.78     | 3.55    | 1.62  | –       | 5.43     | 5.96<sup>a,b</sup> | 4.66  | 1.75  |
| 1.0                    | 2.48     | 3.78    | 1.85  | –       | 4.64     | 5.74<sup>b</sup> | 4.35  | 2.11  |
| **SEM**                | 0.12     | 0.13    | 0.12  | –       | 0.17     | 0.13    | 0.17  | 0.12  |

**Main effect**

| 0.6 | 2.63 | 3.66 | 2.01 | – | 3.73 | 4.76 | 3.49 | 1.65 |
| 0.8 | 2.89 | 3.48 | 2.15 | – | 3.93 | 5.32 | 3.87 | 1.57 |
| 0.9 | 2.67 | 3.49 | 1.80 | – | 4.23 | 5.02 | 3.82 | 1.53 |
| 1.0 | 2.70 | 3.70 | 1.98 | – | 4.00 | 4.94 | 3.42 | 1.72 |

**PBS**

| 2.71 | 3.45 | 2.01 | – | 2.99<sup>b</sup> | 4.15<sup>b</sup> | 2.78<sup>b</sup> | 1.34<sup>b</sup> |
| 2.73 | 3.71 | 1.97 | – | 4.95<sup>a</sup> | 5.87<sup>a</sup> | 4.53<sup>b</sup> | 1.89<sup>a</sup> |

**P-value**

| SID M+C | Challenge | Interaction | | | | | |
|---------|-----------|-------------|--------|--------|--------|--------|--------|
| 0.888   | 0.912     | 0.819       | –      | <0.001 | <0.001 | <0.001 | 0.022 |
| 0.960   | 0.360     | 0.876       | –      | <0.001 | <0.001 | <0.001 | 0.135 |
| 0.755   | 0.882     | 0.372       | –      | 0.434  | 0.067  | 0.643  | 0.874 |

<sup>a,b</sup>Means with different superscripts within a column were significantly different (*P* < 0.05).

<sup>1</sup>Titer was defined as Log 2 of the highest dilution of sample with an optical density equal to the standard intestinal luminal diluted 1:128 (cutoff, two times of the background).

<sup>2</sup>On day 14, broilers were orally gavaged with either PBS or the Advent Coccidiosis Vaccine (Lincoln, NE; 100× vaccine dose; consisting of a blend of live *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* oocysts).

<sup>3</sup>SID M+C, standardized ileal digestible methionine + cysteine; PBS, phosphate-buffered saline.

<sup>4</sup>No anti-*Eimeria* IgA was detected in cecum luminal on day 14.
Table 7. Intestinal luminal total IgA concentrations of broilers fed varying levels of sulfur amino acids in control conditions or challenged with Eimeria spp.

| Challenge | SID M+C (%) | Duodenum | Jejunum | Ileum | Cecum | Duodenum | Jejunum | Ileum | Cecum |
|-----------|-------------|----------|---------|-------|-------|----------|---------|-------|-------|
| PBS       | 0.6         | 45.40    | 111.37  | 93.33 | 3.81  | 10.68    | 28.34   | 33.55 | 8.83  |
|           | 0.8         | 48.34    | 81.32   | 92.49 | 1.94  | 13.03    | 34.81   | 37.36 | 9.22  |
|           | 0.9         | 57.37    | 113.19  | 96.31 | 2.73  | 11.81    | 35.60   | 37.89 | 7.13  |
| Eimeria   | 1.0         | 40.67    | 78.91   | 94.62 | 3.66  | 11.59    | 35.48   | 37.65 | 8.18  |
|           | 0.6         | 36.48    | 78.87   | 61.83 | 2.04  | 13.44    | 35.40   | 47.93 | 9.29  |
|           | 0.8         | 50.41    | 110.02  | 88.01 | 2.91  | 17.41    | 45.03   | 50.03 | 9.37  |
|           | 1.0         | 47.95    | 94.64   | 100.70| 1.94  | 20.53    | 43.03   | 53.63 | 9.18  |
| SEM       |             | 3.03     | 4.40    | 4.19  | 0.30  |          |         |       |       |

Main effect

|          | 0.6         | 40.94    | 95.12   | 77.58 | 2.92  | 12.06    | 31.87   | 40.74 | 9.11  |
|          | 0.8         | 54.38    | 86.04   | 91.50 | 2.14  | 15.22    | 39.92   | 43.70 | 9.30  |
|          | 0.9         | 55.84    | 111.60  | 92.16 | 2.82  | 16.17    | 39.32   | 45.76 | 8.16  |
|          | 1.0         | 44.01    | 87.22   | 97.66 | 2.80  | 15.11    | 39.08   | 44.62 | 8.72  |
| PBS      |             | 47.80    | 96.42   | 94.19 | 3.04  | 11.78    | 33.56   | 36.61 | 8.34  |
| Eimeria  |             | 49.79    | 93.58   | 85.27 | 2.31  | 17.50    | 41.54   | 50.79 | 9.30  |

Interaction

|          | 0.230       | 0.135    | 0.379   | 0.790 | 0.634   | 0.067    | 0.684  | 0.910 |
|          | 0.744       | 0.743    | 0.293   | 0.230 | 0.015   | 0.001    | <0.001 | 0.427 |
|          | 0.604       | 0.242    | 0.430   | 0.419 | 0.803   | 0.957    | 0.986  | 0.949 |

Table 8. Plasma concentrations of IL-10 and IFN-γ of 21-day-old broilers fed varying levels of sulfur amino acids in control conditions or challenged with Eimeria spp.

| Challenge | SID M+C (%) | IL-10 (μg/mL) | IFN-γ (ng/mL) |
|-----------|-------------|---------------|---------------|
| PBS       | 0.6         | 84.76         | 17.60         |
|           | 0.8         | 74.12         | 15.93         |
|           | 0.9         | 72.22         | 33.73         |
| Eimeria   | 1.0         | 82.40         | 18.34         |
|           | 0.6         | 93.28         | 16.29         |
|           | 0.8         | 67.41         | 15.21         |
|           | 0.9         | 86.07         | 16.54         |
|           | 1.0         | 53.93         | 14.84         |
| SEM       |             | 4.48          | 1.96          |

Main effect

|          | 0.6         | 89.02         | 16.95         |
|          | 0.8         | 70.77         | 15.57         |
|          | 0.9         | 79.15         | 25.14         |
|          | 1.0         | 68.16         | 16.50         |
| PBS      |             | 78.38         | 21.40         |
| Eimeria  |             | 75.17         | 15.72         |

P-value

| SID M+C | 0.354 | 0.278 |
| Challenge | 0.722 | 0.146 |
| Interaction | 0.347 | 0.394 |

antibody response (to Newcastle Disease Virus) was higher in broilers fed diets with 0.3 to 0.6% methionine than those fed diets with 0.7 to 1.1% methionine. Similarly, Bhargava et al. (1971) reported that antibody response (to Newcastle Disease Virus) was higher in broilers fed 0.4% methionine than in broilers fed 0.7 and 1.1% methionine. The experimental diets contend no cysteine in the aforementioned Bhargava et al. studies. In this sense, the marginal requirement for SID M+C is narrow in broilers when both growth performance and intestinal humoral immunity are considered (in the current study the range was ≥0.8 and <1.0%). One possible explanation for the lack of observed growth performance and immune response to increasing dietary SID M+C in Eimeria-infected birds could be related to ratios of essential amino acids. By increasing a single amino acid in the feed formulation, we may have inadvertently created a limitation in the another limiting amino acids required for an efficient anti-Eimeria immune response. A recent study looking at single amino acid reductions in Eimeria-infected broilers showed that not only did a reduction in total M+C detrimentally affect growth performance, but reducing threonine, isoleucine, arginine, phenalalnine+tyrosine, and glycine+serine likewise impaired growth performance in challenged birds (Rochell et al., 2016). Follow-up studies by the same group reported arginine specifically may be vital to innate immune responses to Eimeria infection in broilers and be required in higher dietary concentrations in the diets of broilers with coccidiosis infection (Rochell et al., 2017). It would, therefore, be beneficial to conduct further research investigating the interactions of amino acids in the diets of Eimeria-challenged broilers to define not only the single amino acid requirements, but also the ideal ratios of amino acid in challenge conditions.
In the current *Eimeria*-challenged broilers, when compared to broilers fed 0.8% SID M+C, diets with 1.0% SID M+C decreased intestinal anti-*Eimeria* IgA concentrations, but had no adverse effects on growth performance. Broilers fed diets with 0.8% SID M+C had enhanced growth performance and intestinal IgA production compared with broilers fed diets with 0.6% SID M+C, but no differences were observed in intestine lesion scores and excreta oocyst counts. The failure to detect differences in intestinal scores and excreta oocyst counts imply that these *Eimeria* infection indicators do not correlate well with growth performance or intestinal humoral immunity at least when these variables are measured at the same time point, consistent with earlier reports (Brake, 2002; Ding et al., 2004). Additionally, while the 1.0% SID M+C treatment did not show better growth performance than the treatment receiving 0.8% dietary SID M+C, the increase in anti-*Eimeria* antibody titers observed in this group may indicate protective effects and improved performance in a secondary infection. Further research should include studies investigating the effects of dietary sulfur amino acid levels in more than one cycle of coccidiosis.

Intestinal INF-γ is a cytokine which may inhibit the development of parasites (Fayer, 1971) and has been proposed as an important host immunological response during *Eimeria* infection (Yun et al., 2000). Lillehoj and Choi (1998) reported that protein administration of INF-γ protected against weight loss in *Eimeria*-challenged chickens. In the current study, dietary SID M+C levels had no effects on intestinal luminal INF-γ concentrations after *Eimeria* infection. These results suggest the currently observed effects of SID M+C on growth performance and jejunal anti-*Eimeria* IgA levels were not mediated by INF-γ responses. While the anti-parasitic effects of INF-γ may be explained in other aspects of immune function, based on the current results we can exclude humoral antibody production from INF-γ's mechanism of action. The current study also demonstrated dietary SID M+C levels had no effects on intestinal luminal levels of IL-10, an immune suppressor cytokine that may thwart the development of host immunity against viruses, bacteria and helminths (Collier et al., 2008; Cyktor and Joanne, 2011). We have previously shown that an oral antibody to IL-10 can reduce the growth suppression caused by *Eimeria* infection (Arendt et al., 2016; Sand et al., 2016; Raabis et al., 2018). The current results indicate that IL-10 is not involved in the sulfur amino acids-derived effects on growth performance and intestinal anti-*Eimeria* IgA concentrations. Thus, a suitable level of SID M+C and supplementation of anti-IL-10 antibodies together may have additive effects in managing *Eimeria* infection in chickens, and need to be further studied.

In conclusion, when dietary SID M+C was ≥0.8%, the growth suppression caused by *Eimeria* infection could not be further reduced by increasing dietary SID M+C supplementation. For 11- to 21-D broilers, the margin for optimal SID M+C requirement was between 0.8 and 1.0% when considering both growth performance and intestinal humoral immunity. Dietary SID M+C levels had no effect on intestinal luminal production of INF-γ and IL-10. Further studies will investigate the requirement of SID M+C in diets enriched with...
other essential amino acids to further define the ideal protein ratios for amino acids in coccidiosis conditions.

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