Effects of Daily Oral Doses of L-Arginine on Coccidiosis Infections in Chickens

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ABSTRACT L-arginine is an essential amino acid for chickens, as well as the substrate for biosynthesis of nitric oxide (NO), a bioregulatory free radical molecule known to have antimicrobial activity. Biosynthesis of NO by induced nitric oxide synthase (iNOS) can be stimulated during immunological response to infection. Therefore, in chickens, production of NO as a response to an infection should be influenced by dietary levels of L-arginine. Two experiments were carried out to determine whether oral dosing with L-arginine during coccidia infections could influence the associated pathology or the development of the parasites. Neither single nor dual daily doses (500 mg/kg) of L-arginine reversed weight gain reduction, augmented plasma NO$_2^-$ + NO$_3^-$ levels, or lessened lesion scores in chicks infected with Eimeria acervulina, Eimeria maxima, or Eimeria tenella. Although the oocyst shedding from E. maxima (1x dose) or E. acervulina (2x dose) infections were not affected, the oocyst shedding from E. tenella infections was significantly reduced by both dose regimens.

(Key words: coccidiosis, dietary L-arginine, nitric oxide)

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INTRODUCTION

Reduced weight gain is a major contributor to the production losses that accompany coccidia infections in young chickens. Because inflammatory immune responses divert energy from growth (Klasing et al., 1987), they may adversely affect weight gain. Production of nitric oxide (NO) as a result of infection can be considered part of the inflammatory response because it is most often triggered by inflammatory cytokines such as interferon gamma, interleukin-1, or tumor necrosis factor-alpha (Stuehr and Marletta, 1987).

Plasma levels of the ions NO$_2^-$ and NO$_3^-$, stable metabolites of NO, have been used together (Stuhler and Marletta, 1985; Nussler et al., 1994; Prada and Kremsner, 1995) as an index of in vivo NO responses to infection, and it has been shown that plasma levels of NO$_2^-$ + NO$_3^-$ increase significantly during primary infections with Eimeria acervulina (Allen and Teasdale, 1994), Eimeria tenella (Allen, 1997a), and Eimeria maxima (Allen, 1997b), although these responses are not as great as those seen in infection models with other animals such as rodents (Evans et al., 1994; Grisham et al., 1994; Vespa et al., 1994).

Although NO production has been shown to be important to host defense against intracellular parasites such as Trypanosoma brucei, Toxoplasma gondii, Plasmodium berghei, and Plasmodium falciparum (MacMicking et al., 1997), this relationship has not been established for Eimeria infections in chickens, although there is some evidence for the association of genetic resistance with a heightened in vivo NO$_2^-$ + NO$_3^-$ response (Allen and Lillehoj, 1998).

The enzyme thought to be responsible for high and sustained output of NO during an immune response is induced nitric oxide synthase (iNOS) from activated macrophages (Xie and Nathan, 1994), the substrate for which is L-arginine (Moncada and Higgs, 1993). Because chickens cannot biosynthesize arginine via the ornithine cycle, they must obtain this essential amino acid directly from the diet (Tamir and Ratner, 1963; Boorman and Lewis, 1971). Therefore, in theory, the NO response to infection and its subsequent effects on the host and infecting organism should be manipulatable with dietary L-arginine. If NO is toxic to Eimeria sp., oral administration of L-arginine might be expected to improve weight gain, augment the plasma NO$_2^-$ + NO$_3^-$ response, and decrease lesion scores and oocyst shedding with respect to untreated controls. This report summarizes results of trials in which coccidia-infected chickens consuming a commercial broiler starter ration were treated orally with one or two daily doses of L-arginine over the periods of infection.
MATERIALS AND METHODS

Chickens and Housing

Day-old male Sex Sal chicks were raised in Brower brooders until 3 wk of age, at which time they were wing-banded, weighed, and transferred to suspended wire cages. Five chicks were housed per cage, in rooms with constant lighting, maintained between 25 and 28 C. Chicks were allowed free access to water and a broiler starter ration (BSR). During periods of coccidia infection, the infected and uninfected chickens were housed in separate buildings.

Parasites and Infections

Laboratory strains of E. acervulina (strain 12), E. maxima (strain ES), and E. tenella (strains 80 and MS) were used. These strains were purified by single oocyst isolation and propagated by periodic passage through chickens at the Parasite Biology and Epidemiology Laboratory (Beltsville, MD). Experimental infections were initiated in each chicken by administering by gavage a designated number of oocysts in 1 mL water.

Experiment 1

At 3 wk of age, chickens were divided into six groups having equivalent mean weight, with 20 chicks per group. Chickens were housed five per cage. Chickens in three groups were given daily doses of L-arginine (500 mg/kg, 0800 and 1600 h) by gavage beginning 1 d prior to infection with coccidia through 8 d PI. Chickens in one treated and one untreated group were then each inoculated with 500,000 sporulated oocysts of E. acervulina or 25,000 sporulated oocysts of E. tenella strain MS. Chickens in one treated and one untreated group remained uninfected controls. At 6 d PI, weight gains were determined, and 10 chickens per group were bled, euthanized, and scored for lesions. Fecal samples were collected from remaining infected groups from Days 5 to 9 PI for oocyst shedding determination.

Analyses

Plasma samples, which had been frozen following collection, were thawed and filtered through Centricon-30 filters to obtain clear, colorless filtrates. These were diluted 1:3 with distilled water, and total NO \(_3^-\) + NO \(_2^-\) in 150 \(\mu\)L of the diluates was analyzed colorimetrically using Griess reagents after reducing the NO \(_3^-\) to NO \(_2^-\) with nitrate reductase according to Verdon et al. (1995). Analyses were carried out in 96-well microtiter plates. The differences in absorbances at 540 nm (peak) and 630 nm (baseline) were proportional to NO \(_2^-\) concentration in the range from 0.0325 to 50 \(\mu\)M.

Oocyst shedding was determined from duplicate counts of duplicate samplings of homogenates from duplicate collections per infected treatment group using a McMasters type chamber (Conway and McKenzie, 1991) and expressed as oocysts shed per chick.

Statistics

Data were statistically analyzed using the General Linear Models program of SAS (SAS Institute, 1990), and differences (\(P \geq 0.05\)) among treatment group means were determined using Duncan’s multiple range test.

RESULTS

Experiment 1

Regardless of treatment with L-arginine, infection with E. tenella significantly reduced weight gain during the 6 d of infection. Infection with E. maxima or E. tenella significantly increased plasma NO \(_3^-\) + NO \(_2^-\) (Table 1). However, within an infection group, treatment with a single daily dose of L-arginine had no significant effect on weight gain at 6 d PI, plasma NO \(_3^-\) + NO \(_2^-\), or lesion scores (Table 1). Oocyst shedding from E. maxima-infected chickens was not significantly affected by L-arginine treatment. However, it was significantly reduced (26%) in E. tenella-infected chickens (Table 1).

Experiment 2

Regardless of L-arginine treatment, infection with either E. acervulina or E. tenella significantly decreased in three groups were given two daily doses of L-arginine (500 mg/kg, 0800 and 1600 h) by gavage beginning 1 d prior to infection with coccidia through 8 d PI. Chickens in one treated and one untreated group were then each inoculated with 500,000 sporulated oocysts of E. acervulina or 25,000 sporulated oocysts of E. tenella strain MS. Chickens in one treated and one untreated group remained uninfected controls. At 6 d PI, weight gains were determined, and 10 chickens per group were bled, euthanized, and scored for lesions. Fecal samples were collected from remaining infected groups from Days 5 to 9 PI for oocyst shedding determination.
weight gain and increased plasma NO\textsubscript{2} + NO\textsubscript{3} (Table 2). Within an infection group, two daily doses of 500 mg/kg with L-arginine spaced 8 h apart had no significant effects on weight gain at 6 d PI, plasma NO\textsuperscript{−} + NO\textsubscript{3}, or lesion scores (Table 2). Oocyst shedding from E. acervulina-infected chickens was not significantly affected by the L-arginine treatment. However, the L-arginine significantly reduced (39%) oocyst shedding from E. tenella-infected chickens (Table 2).

**DISCUSSION**

The goals of the experiments in this report were to determine whether four variables associated with the pathology caused by coccidia infections, namely, weight gain, plasma NO\textsubscript{2} + NO\textsubscript{3}, lesion score, and oocyst shedding, could be influenced by oral administration of L-arginine, the substrate for the enzyme iNOS. Further, it was hoped that some relationship between NO production and development of the parasite, as judged from oocyst shedding, could be inferred from the results.

The lack of a sparing effect by oral doses of L-arginine on weight gain of infected chickens suggests that the availability of this essential amino acid was not a rate-limiting factor for growth in infected chickens. Additionally, the lack of augmentation of the plasma NO\textsubscript{2} + NO\textsubscript{3} levels that are normally elevated at 6 d PI (Tables 1 and 2) suggested that sufficient L-arginine substrate for iNOS activity was provided in the diet of infected control groups.

Lack of effect of supplemental L-arginine on gross lesion scores may reflect the subjectivity of the scoring procedure, which, although generally dependent on the extent of mucosal invasion, can be complicated by host inflammatory responses such as mucus and exudate production and bleeding.

Both single and dual doses of L-arginine significantly reduced oocyst shedding from E. tenella infections, but not E. acervulina or E. maxima infections. Despite the lack of effect on lesion scores, supplementary L-arginine appeared to aid in inhibiting development of E. tenella. One interpretation of these observations is that supplemental L-arginine can overcome substrate limiting rates of NO production at local sites of infection and that the early stages of E. tenella development (merogony) around Days 3 and 4 PI may be more susceptible to the consequences of NO production than those of E. acervulina or E. maxima.

**TABLE 1. Effects of single daily doses of L-arginine (500 mg/kg) on Eimeria maxima and Eimeria tenella infections at 6 d postinoculation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Uninfected</th>
<th>E. maxima</th>
<th>E. tenella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain, g</td>
<td>Untreated</td>
<td>91 ± 3\textsuperscript{a}</td>
<td>90 ± 2\textsuperscript{b}</td>
<td>49 ± 5\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>90 ± 3\textsuperscript{a}</td>
<td>94 ± 3\textsuperscript{a}</td>
<td>46 ± 5\textsuperscript{a}</td>
</tr>
<tr>
<td>Plasma NO\textsubscript{2} + NO\textsubscript{3}, μM</td>
<td>Untreated</td>
<td>11.1 ± 0.4\textsuperscript{d}</td>
<td>15.0 ± 0.4\textsuperscript{b}</td>
<td>31.0 ± 1.7\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>10.6 ± 0.5\textsuperscript{c}</td>
<td>15.2 ± 0.7\textsuperscript{b}</td>
<td>32.8 ± 1.5\textsuperscript{a}</td>
</tr>
<tr>
<td>Lesion scores\textsuperscript{1}</td>
<td>Untreated</td>
<td>0</td>
<td>1.5 ± 0.2\textsuperscript{a}</td>
<td>3.5 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>1.2 ± 0.3\textsuperscript{a}</td>
<td>3.2 ± 0.3\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Oocysts shed per chick, \times10\textsuperscript{6}</td>
<td>Untreated</td>
<td>0</td>
<td>17.0 ± 1.4\textsuperscript{a}</td>
<td>81.0 ± 9.9\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>14.7 ± 7.5\textsuperscript{a}</td>
<td>60.0 ± 3.6\textsuperscript{a}</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a-c}Means ± SEM within a variable with no common superscript differ (P ≥ 0.05).

\textsuperscript{a,b}Means ± SEM within columns with no common superscript differ (P ≥ 0.05).

\textsuperscript{1}Scores range from 0 to 4, indicating no lesions to severe lesions, respectively.

\textsuperscript{2}Oocysts collected from 5 to 9 d postinoculation.

**TABLE 2. Effects of two daily doses of L-arginine (500 mg/kg) on Eimeria acervulina and Eimeria tenella infections at 6 d postinoculation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Uninfected</th>
<th>E. acervulina</th>
<th>E. tenella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gains, g</td>
<td>Untreated</td>
<td>114 ± 4\textsuperscript{a}</td>
<td>74 ± 6\textsuperscript{b}</td>
<td>53 ± 10\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>116 ± 2\textsuperscript{a}</td>
<td>63 ± 6\textsuperscript{c}</td>
<td>46 ± 7\textsuperscript{a}</td>
</tr>
<tr>
<td>Plasma NO\textsubscript{2} + NO\textsubscript{3}, μM</td>
<td>Untreated</td>
<td>10.1 ± 0.6\textsuperscript{b}</td>
<td>27.8 ± 3.3</td>
<td>26.6 ± 1.1\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>10.7 ± 0.5\textsuperscript{a}</td>
<td>24.3 ± 2.4</td>
<td>25.8 ± 2.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Lesion scores\textsuperscript{1}</td>
<td>Untreated</td>
<td>0</td>
<td>3.6 ± 0.3\textsuperscript{a}</td>
<td>3.6 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0</td>
<td>4.0 ± 0\textsuperscript{c}</td>
<td>3.5 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Oocysts shed per chick, \times10\textsuperscript{6}</td>
<td>Untreated</td>
<td>0</td>
<td>4.0 ± 0.4\textsuperscript{a}</td>
<td>1.8 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>5.0 ± 0.4\textsuperscript{a}</td>
<td>1.1 ± 0.1\textsuperscript{a}</td>
<td></td>
</tr>
</tbody>
</table>
Analysis of the BSR provided by Southern States Co. shows a percentage of 1.6418 for L-arginine. This level is somewhat higher than NRC recommendations (1994). The daily L-arginine intake of control chickens from BSR during the 6-d infection period is roughly estimated to be 0.547 g (assuming 100 g overall gain and a feed conversion of about 2). Assuming an average chick weight of about 300 g, a 1x oral dose of L-arginine would provide an additional daily 0.150 g, and a 2x oral dose, 0.300 g, so that the estimated daily L-arginine intake per chick would be 0.547, 0.697, and 0.847 g, for untreated, 1x-treated, and 2x-treated chicks, respectively.

Taylor et al. (1992) found that, compared with a low dietary level (0.92%) of L-arginine, a high dietary level (2.4%) reduced the tumor scores (based on tumor size) by about 24% in chicks experimentally infected with subgroup A Rous sarcoma virus. This study was based on the assumption derived from in vitro studies that high dietary L-arginine would result in elevated NO production that, in turn, would be toxic to the virus-induced tumors. However, these authors also found no effect of increased dietary L-arginine on weight gain or on the tumors. However, these authors also found no effect of increased dietary L-arginine on weight gain or on the latent period for tumor development.

For comparison, estimates of daily L-arginine intake based on dietary levels used by Taylor et al. (1992) would have been, in the present experiment, 0.306 g L-arginine for the basal diet and 0.799 g for the high diet. Their basal level, which was considered quite adequate for growth, was 0.306 g L-arginine compared with the BSR, and might have been truly rate-limiting with respect to providing sufficient substrate for iNOS generated in their experimental model.

In summary, because chickens require L-arginine as an essential amino acid, the control and study of physiological effects of metabolic pathways for which L-arginine is a substrate, such as NO synthesis by macrophage iNOS, should be possible through dietary manipulation with L-arginine. However, it may not be possible to show significant effects if basal diets provide levels higher than about 1%. This conclusion seems to be particularly true for the study of avian coccidiosis in which NO responses to primary infection are relatively low compared with those seen in rodents (Evans et al., 1994; Grisham et al., 1994; Vespa et al., 1994). Further studies using basal diets with low L-arginine levels are planned.

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REFERENCES


