Ascorbic Acid Supplementation Improved Antibody Response to Infectious Bursal Disease Vaccination in Chickens

J. Amakye-Anim,*,1 T. L. Lin,* P. Y. Hester,† D. Thiagarajan,‡ B. A. Watkins,§ and C. C. Wu*2

*Department of Veterinary Pathobiology, †Department of Animal Science, ‡Animal Disease Diagnostic Laboratory, and §Department of Food Science, Lipid Chemistry and Molecular Biology Laboratory, Purdue University, West Lafayette, Indiana 47907-1175

ABSTRACT The purpose of the present study was to determine if supplementation of ascorbic acid (AA) to the diet would have a beneficial effect on infectious bursal disease (IBD) vaccination of chickens for protection against infectious bursal disease virus (IBDV) infection. Two hundred forty specific pathogen-free (SPF) chickens were divided into eight experimental groups. A 2×2×2 factorial arrangement in a completely randomized design was used; AA supplementation at 1,000 ppm in the diet, vaccination, and challenge were the main effects. Prior to challenge and 10 d after challenge, serum AA concentration, serum corticosterone concentration, ELISA antibody titer to IBDV, body weight, bursa-to-body weight (B:B) ratio, and bursal histological score (BHS) were determined. Nonvaccinated chickens fed a diet supplemented with AA did not exhibit clinical signs or mortality following challenge, whereas AA-unsupplemented counterparts had 100% cumulative morbidity and 30% cumulative mortality. Serum AA levels of AA-supplemented and vaccinated chickens were significantly (P < 0.05) higher than AA-unsupplemented and vaccinated chickens. Fourteen days following vaccination, significantly (P < 0.05) higher ELISA titers to IBDV were observed in vaccinated chickens supplemented with AA as compared to AA-unsupplemented counterparts. Ascorbic acid-supplemented chickens, especially those also vaccinated, had higher body weight gains as compared to the AA-unsupplemented chickens. Ascorbic acid-supplemented chickens challenged with IBDV did not show any clinical signs or mortality. The results suggest that supplementation of AA at 1,000 ppm in the diet has beneficial effects on antibody response to IBD vaccination and body weight gain.

(Key words: ascorbic acid, vitamin C, infectious bursal disease, infectious bursal disease virus)

INTRODUCTION

Infectious bursal disease (IBD) caused by infectious bursal disease virus (IBDV) is an acute contagious viral disease of chickens, resulting in bursal lymphocytolysis and subsequent immunosuppression. It remains a major contributor to economic loss in the poultry industry. Humoral immunity is the primary mechanism of the protective immune response for IBDV infection (Wood et al., 1981). The principal method for the control of IBDV is by vaccination. Hens are hyperimmunized so that they will transmit high levels of maternal antibody to their progeny for the first 2 to 3 wk after hatch. However, the level of passive immunity is variable and unpredictable. Therefore, a common commercial practice is to vaccinate all chickens against IBDV with a live attenuated vaccine during the first 3 wk posthatch (Winterfield et al., 1980). Furthermore, chickens vaccinated with classical serotype 1 IBD vaccine are not protected against infection with variant viruses (Saif, 1994). An ideal live attenuated IBD vaccine should not cause disease or bursal lesions, not be immunosuppressive, and not be excreted but should stimulate a long-lasting immunity and protect chickens from being infected by classical and variant IBDV strains. However, such a vaccine is not available. Thus, other economical and effective ways are being investigated to increase protection against IBDV infection in chickens.

Ascorbic acid (AA) has been demonstrated to improve immunoresponsiveness and increase disease resistance in chickens by optimizing the functions of the immune system (Pardue et al., 1985; Rund, 1989). In the first line

Received for publication July 23, 1999.
Accepted for publication February 7, 2000.
1Present address: Veterinary Services Department, PO Box M161, Accra, Ghana, West Africa.
2To whom correspondence should be addressed: 1175 Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN 47907; e-mail: wuc@purdue.edu.

Abbreviation Key: AA = ascorbic acid; B:B = bursa-to-body weight; BHS = bursal histopathological score; IBD = infectious bursal disease; IBDV = infectious bursal disease virus; SPF = specific pathogen-free; STC = standard challenge strain of infectious bursal disease virus.
of defense against pathogens, phagocytosis by neutrophils involves increased consumption of both ascorbate and dehydroascorbate (Stankova et al., 1975; Rund, 1989). In addition, viral infections have been shown to cause depletion of leukocyte ascorbate, resulting in varying degrees of nonspecific immunosuppression (Thomas and Holt, 1978). Ascorbic acid can modulate the activity of B cells, and addition of dietary ascorbate prior to immunization has been found to increase antibody production (McCorkle et al., 1980). Dietary supplementation with AA, therefore, may have beneficial effects on immunoresponsiveness in chickens.

We considered the occurrence of IBD despite intensive IBD vaccination programs and the potential advantage of dietary supplementation of AA to improve immune responsiveness and disease resistance. Thus, the objective of the present study was to determine if the inclusion of supplemental AA to the diet would have a beneficial effect on IBD vaccination of chickens for protection against IBDV infection.

**MATERIALS AND METHODS**

**Experimental Design**

Two hundred forty specific pathogen-free (SPF) chickens were individually weighed, wing-banded, and randomly distributed to eight experimental groups. A 2 \( \times \) 2 factorial arrangement in a completely randomized design was used; AA supplementation, vaccination, and challenge were the main effects. Chickens in experimental Groups 1, 2, 3, and 4 were fed a diet not supplemented with AA (Diet B), whereas chickens in Groups 5, 6, 7, and 8 were fed a diet supplemented with AA at 1,000 ppm (Diet A) from hatching to 31 d of age. Chickens in Groups 1, 2, 5, and 6 were orally vaccinated with commercial classical vaccine at 7 d of age. Chickens in Groups 1, 2, 3, and 4 were fed Diets A or B (Table 1), formulated according to Nutrient Requirements of Poultry provided by the National Research Council (National Research Council, 1994), from hatching to 31 d of age. Feed and water were provided ad libitum. Ascorbic acid in ethylcellulose-coated form (97.5% purity) was used.

**Chickens and Diets**

The chickens, divided into eight experimental groups, were reared in separate Horsfall isolation units. Chickens were fed Diets A or B (Table 1), formulated according to Nutrient Requirements of Poultry provided by the National Research Council (National Research Council, 1994), from hatching to 31 d of age. Feed and water were provided ad libitum. Ascorbic acid in ethylcellulose-coated form (97.5% purity) was used.

**Vaccine**

Commercial classical virus vaccine, Bursine II was used. Chickens in Groups 1, 2, 5, and 6 were orally inoculated with Bursine II at 50 \( \mu \)L per chicken at 7 d of age.

**Challenge Virus**

Bursa-derived STC strain of serotype 1 IBDV was used as the challenge virus. The concentration of the virus in the bursal homogenate was titrated by inoculating 10-d-old SPF embryonating eggs via chorioallantoic membrane (Hitchcock, 1970). A 1 \( \times \) 10^{3.2} 50% embryo lethal dose of STC virus was given to chickens in Groups 1, 3, 5, and 7 orally at 21 d of age.

**Determination of Serum AA**

Serum AA concentration was determined by a spectrophotometric method based on the reduction of tetrazolium salt by AA (Beutler, 1984) according to the procedures recommended by the manufacturer. One milliliter

---

3SPAFAS Inc., Roanoke, IL 61561.
4Hoffman La Roche Inc., Nutley, NJ 07110.
6Boehringer Mannheim, Indianapolis, IN 46290.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal</td>
<td>A = 35.00 B = 35.00</td>
</tr>
<tr>
<td>Ground yellow corn</td>
<td>A = 54.05 B = 54.05</td>
</tr>
<tr>
<td>Corn starch</td>
<td>A = 1.0 B = 1.0</td>
</tr>
<tr>
<td>Lipid</td>
<td>A = 3.0 B = 3.0</td>
</tr>
<tr>
<td>CaHPO4 ( \times )2H2O</td>
<td>A = 2.0 B = 2.0</td>
</tr>
<tr>
<td>CaCO3</td>
<td>A = 1.2 B = 1.2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>A = 0.25 B = 0.25</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>A = 3.0 B = 3.0</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>A = 0.5 B = 0.5</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>A = 0.1 B = 0.0</td>
</tr>
</tbody>
</table>

A = diet with supplementation of ascorbic acid at 1,000 ppm; B = diet without ascorbic acid supplementation.
of serum sample was used in the test, and the results were read at 578 nm in a spectrophotometer.7

**Determination of Serum Corticosterone**

Serum corticosterone levels were determined by a solid-phase radioimmunoassay, Coat-A-Count procedure,6 based on competitive binding of 125I-labeled corticosterone to a high affinity antibody (Al-Dajaili et al., 1981). One hundred microliters of 1:2 PBS-diluted serum sample from each chicken were used in the assay. Corticosterone levels in serum samples were measured using a standard curve established by measuring 125I-labeled corticosterone with a gamma counter.

**Determination of Antibody Titer to IBDV**

Antibody titer to IBDV was determined by an ELISA kit9 (Snyder et al., 1984), according to manufacturer’s instructions. One hundred microliters of serum from each chicken were used in the assay. The results were read at 650 nm in a microplate reader.10

**Calculation of Bursa-to-Body Weight Ratio**

Individual bursa-to-body weight (B:B) ratio was calculated by dividing bursal weight by body weight and multiplying by 1,000 (Giambrone and Closser, 1990).

**Histopathological Examination**

Bursae were fixed in 10% formalin and processed for hematoxylin- and eosin-stained sections. Bursal sections were examined under a light microscope. Bursal lesions were evaluated using a scale from 1 to 4 with 4 being the most severe (Henry et al., 1980). Bursa with a range of 0 to 10% follicular atrophy were scored 1, 10 to 30% follicular atrophy were scored 2, 30 to 70% follicular atrophy were scored 3, and 70 to 100% follicular atrophy were scored 4.

**Statistical Methods**

The data were subjected to ANOVA and t-test procedures. Statements of statistical significance were based on $P < 0.05$. Comparisons among groups were made using Duncan’s multiple-range test in which groups were more than two (Snedecor and Cochran, 1980), and t-test was used in comparing two major groupings (Myra, 1989). All tests were performed using SAS Software (SAS Institute Inc., 1989).

---

7 Hewlett Packard, Palo Alto, CA 94304.
8 Diagnostic Products Corporation, Los Angeles, CA 90016.
9 IDEXX Inc., Westbrook, ME 04092.
10 Molecular Devices, Sunnyvale, CA 94089.

**RESULTS**

**Clinical Signs**

Clinical signs of anorexia, dullness, incoordination, wet vents, and ruffled feathers were observed 2 to 3 d postchallenge in chickens (Group 3) fed a diet not supplemented with AA and not vaccinated with IBD vaccine but challenged with IBDV strain STC. Signs disappeared 6 to 7 d postchallenge. Cumulative morbidity was 100%, and cumulative mortality was 30% by 10 d postchallenge at the end of experiment. Chickens (Groups 5, 6, 7, and 8) fed the diet supplemented with AA, with or without vaccination or challenge, did not exhibit any clinical signs or mortality. No clinical signs or mortality was observed in chickens in the negative control (Group 4) or in those not supplemented with AA but vaccinated with IBD vaccine (Groups 1 and 2).

**Serum AA Levels**

Prior to vaccination (7 d old), serum AA levels of chickens (Groups 5, 6, 7, and 8) fed diet supplemented with AA were significantly ($P < 0.05$) higher than those of chickens (Groups 1, 2, 3, and 4) fed the diet not supplemented with AA (Table 2). At 14 d postvaccination and prior to challenge (21 d old), serum AA levels of chickens (Groups 5 and 6) fed the diet supplemented with AA were significantly ($P < 0.05$) higher than those of chickens (Groups 1 and 2) fed the diet without AA-supplementation. Chickens (Groups 7 and 8) fed the diet supplemented with AA and not vaccinated with IBD vaccine had serum AA levels significantly ($P < 0.05$) higher than those of chickens (Groups 3 and 4) fed the diet without supplementation of AA and not vaccinated with IBD vaccine. At 10 d postchallenge (31 d old), significantly ($P < 0.05$) higher serum AA levels were observed in chickens fed the diet supplemented with AA than those fed the diet not supplemented with AA. In chickens (Groups 1, 2, 3, and 4) not supplemented with AA, there was no significant ($P < 0.05$) difference in serum AA levels between chickens vaccinated with IBD vaccine, with or without challenge with the IBDV strain STC (Groups 1 and 2). Chickens (Group 3) not vaccinated with IBD vaccine and challenged with IBDV strain STC had the lowest serum AA level (2.39 ± 0.36 g/mL). Among AA-supplemented chickens (Groups 5, 6, 7, and 8), chickens receiving IBD vaccine only (Group 6) or vaccinated with IBD vaccine and challenged with IBDV strain STC (Groups 5) had similar serum AA levels when compared to those of chickens in Group 8. Chickens (Group 7) not vaccinated with IBD vaccine but challenged with IBDV strain STC (Group 7) had the lowest serum AA level (10.66 ± 2.16 g/mL) when compared to that of chickens in Groups 5, 6, and 8 ($P < 0.05$). However, serum AA level of chickens in Group 7 was significantly ($P < 0.05$) higher than that of chickens in Group 3 fed the AA unsupplemented diet, not vaccinated with IBD vaccine, but challenged with IBDV strain STC.
Prior to vaccination (7 d old), no significant (P < 0.05) differences in serum corticosterone levels were observed between chickens (Groups 5, 6, 7, and 8) supplemented with AA and those (Groups 1, 2, 3, and 4) without AA supplementation (Table 3). At 14 d postvaccination and prior to challenge (21 d old), no significant (P < 0.05) difference in serum corticosterone levels was observed between chickens (Groups 5 and 6) fed the diet supplemented with AA and chickens (Groups 1 and 2) fed the diet not supplemented with AA. Chickens (Groups 7 and 8) fed the diet supplemented with AA but not vaccinated with IBD vaccine had a serum corticosterone level significantly (P < 0.05) higher than that of chickens (Groups 3 and 4) that were fed the diet not supplemented with AA. At 10 d postchallenge mean serum corticosterone levels were significantly (P < 0.05) higher in AA-supplemented and IBDV-challenged chickens (Group 7) than in chickens fed the diet not supplemented with AA, vaccinated

TABLE 3. Serum corticosterone levels (mean ± standard deviation) of chickens with treatments of dietary supplementation with ascorbic acid, vaccination for infectious bursal disease, and challenge with infectious bursal disease virus, alone or in combination, at 7, 21, and 31 d of age

<table>
<thead>
<tr>
<th>Group</th>
<th>AA1</th>
<th>Vaccination2</th>
<th>Challenge3</th>
<th>Serum corticosterone (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age (d)</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>15.18 ± 2.72 ±5</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>16.33 ± 3.71 ±7</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>16.33 ± 3.71 ±7</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>27.47 ± 9.49 ±5</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>15.83 ± 3.73 ±5</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>19.78 ± 4.64 ±9</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>15.96 ± 2.30 ±9</td>
</tr>
</tbody>
</table>

**Values in a column with no common superscript differ significantly (P < 0.05).**

1AA = supplementation of diet with ascorbic acid at 1,000 ppm from 1 to 31 d of age.
2Vaccination with infectious bursal disease vaccine at 7 d of age.
3Challenge with infectious bursal disease virus strain standard challenge strain at 21 d of age.
4Mean serum corticosterone levels for chickens in Groups 1, 2, 3, and 4 at 7 d of age prior to vaccination.
5Mean serum corticosterone levels for chickens in Groups 5, 6, 7, and 8 at 7 d of age prior to vaccination.
6Mean serum corticosterone levels for chickens in Groups 1 and 2 at 21 d of age before challenge.
7Mean serum corticosterone levels for chickens in Groups 3 and 4 at 21 d of age before challenge.
8Mean serum corticosterone levels for chickens in Groups 5 and 6 at 21 d of age before challenge.
9Mean serum corticosterone levels for chickens in Groups 7 and 8 at 21 d of age before challenge.
with IBD vaccine, and challenged with IBDV (Group 1). Decreases in serum corticosterone levels with age were, however, observed in chickens from all the treatment groups.

**ELISA Titer to IBDV**

Before vaccination (7 d old), no \( (P < 0.05) \) significant differences in ELISA titers were observed between chickens (Groups 5, 6, 7, and 8) fed the diet supplemented with AA and chickens fed the diet not supplemented with AA (Groups 1, 2, 3, and 4) (Table 4). Chickens vaccinated with IBD vaccine at 7 d of age had significantly \( (P < 0.05) \) increased ELISA titers at 14 d after vaccination and prior to challenge (21 d old). ELISA titers of chickens in Groups 5 and 6 fed the diet supplemented with AA were significantly \( (P < 0.05) \) higher than those of chickens in Groups 1 and 2 fed the AA-unsupplemented diet.

**Mean Body Weight**

No significant \( (P < 0.05) \) differences in body weight were observed among all groups of chickens at hatch (Table 5). Prior to vaccination (7 d old), mean body weights of chickens fed the diet supplemented with AA (Groups 5, 6, 7, and 8) were significantly \( (P < 0.05) \) higher than those of chickens fed the diet without supplementation of AA (Groups 1, 2, 3, and 4). At 14 d postvaccination and prior to challenge (21 d old), the mean body weight of chickens that were not supplemented with AA and not vaccinated with IBD vaccine (Groups 3 and 4) was significantly \( (P < 0.05) \) lower than that of chickens fed the diet supplemented with AA but not vaccinated with IBD vaccine (Groups 7 and 8). At 10 d postchallenge (31 d old), the mean body weight of chickens in Group 6 receiving AA supplementation and vaccinated with IBD vaccine was significantly \( (P < 0.05) \) higher than that of chickens in Group 2, which was not supplemented with AA but vaccinated with IBD vaccine. The mean body weight (360 g) of chickens in Group 6 was 18% heavier than that (292 g) of chickens in Group 2. No significant \( (P < 0.05) \) difference in mean body weight was observed between chickens in Groups 1 and 5 fed the diet not supplemented or supplemented with AA, respectively, vaccinated with IBD vaccine, and challenged with IBDV strain STC. However, the mean body weight (361 g) of chickens in Group 5 was 15% heavier than that (305 g) of chickens in Group 1. In addition, the mean body weight (338 g) of chickens in Group 8 receiving AA supplementation was only 10% heavier than that (304 g) of chickens in Group 4 which were fed the AA-unsupplemented diet.

**Bursal Histopathological Scores and B:B Ratio**

At 14 d postvaccination, and before challenge (21 d old), no significant \( (P < 0.05) \) differences in bursal histopathological scores (BHS) were observed among the groups of experimental chickens (Table 6). However, chickens fed the diet supplemented with or not supplemented with AA and vaccinated with IBD vaccine had significantly \( (P < 0.05) \) lower B:B ratios than those of chickens fed the diet supplemented with or without AA, but not vaccinated with IBD vaccine. At 10 d postchallenge (31 d old), chickens (Groups 3 and 7) fed the diet supplemented with or not supplemented with AA, not vaccinated with IBD vaccine, but challenged with IBDV strain STC had the lowest B:B ratios and the highest BHS.

---

**TABLE 4. Mean ELISA titers to infectious bursal disease virus in chickens before vaccination (7 d of age) and 14 d after vaccination (21 d of age) with infectious bursal disease vaccine**

<table>
<thead>
<tr>
<th>Group</th>
<th>AA(^1)</th>
<th>Vaccination(^2)</th>
<th>Challenge(^3)</th>
<th>Mean ELISA titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>3(^4)</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>1(^6)</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>3(^7)</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>2(^5)</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>1,161(^8)</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>3(^9)</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

\(^{**}\)Means in a column with no common superscript differ significantly \( (P < 0.05) \).

\(^{1}\)AA = supplementation of diet with ascorbic acid at 1,000 ppm from 1 to 31 d of age.

\(^{2}\)Vaccination with infectious bursal disease vaccine at 7 d of age.

\(^{3}\)Challenge with infectious bursal disease virus strain standard challenge strain at 21 d of age.

\(^{4}\)Mean ELISA titers for chickens in Groups 1, 2, 3, and 4 at 7 d of age prior to vaccination.

\(^{5}\)Mean serum ELISA titers for chickens in Groups 5, 6, 7, and 8 at 7 d of age prior to vaccination.

\(^{6}\)Mean ELISA titers for chickens in Groups 3 and 4 at 21 d of age before challenge.

\(^{7}\)Mean ELISA titers for chickens in Groups 1 and 2 at 21 d of age before challenge.

\(^{8}\)Mean ELISA titers for chickens in Groups 5 and 6 at 21 d of age before challenge.

\(^{9}\)Mean ELISA titers for chickens in Groups 7 and 8 at 21 d of age before challenge.
TABLE 5. Mean body weight (g) of chickens with treatments of dietary supplementation with ascorbic acid, vaccination for infectious bursal disease, and challenge with infectious bursal disease virus, alone or in combination, at 7, 21, and 31 d of age

<table>
<thead>
<tr>
<th>Group</th>
<th>AA</th>
<th>Vaccination</th>
<th>Challenge</th>
<th>1</th>
<th>7</th>
<th>21</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>39.6a</td>
<td>55.72b,4</td>
<td>180.58a,6</td>
<td>305.0abc</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>39.5a</td>
<td>292.0bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>38.4a</td>
<td>171.35b,7</td>
<td>361.0a</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>38.9a</td>
<td>59.46a,5</td>
<td>304.0abc</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>39.0a</td>
<td>178.40a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>39.7a</td>
<td>360.0a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>40.0a</td>
<td>178.05a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>39.7a</td>
<td>338.0a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Means in a column with no common superscript differ significantly (*P* < 0.05).

1AA = supplementation of diet with ascorbic acid at 1,000 ppm from 1 to 31 d of age.
2Vaccination with infectious bursal disease vaccine at 7 d of age.
3Challenge with infectious bursal disease virus strain standard challenge strain at 21 d of age.
4Mean body weight for chickens in Groups 1, 2, 3, and 4 at 7 d of age prior to vaccination.
5Mean body weight for chickens in Groups 5, 6, 7, and 8 at 7 d of age prior to vaccination.
6Mean body weight for chickens in Groups 1 and 2 at 21 d of age before challenge.
7Mean body weight for chickens in Groups 3 and 4 at 21 d of age before challenge.
8Mean body weight for chickens in Groups 5 and 6 at 21 d of age before challenge.
9Mean body weight for chickens in Groups 7 and 8 at 21 d of age before challenge.

(score = 4). Microscopically, more than 75% of the bursal lymphoid follicles were markedly depleted of lymphocytes and atrophied. Chickens in Group 6 fed the diet supplemented with AA, vaccinated with IBD vaccine, but not challenged with IBDV strain STC had a significantly (*P* < 0.05) higher B:B ratio than that of chickens in Group 2 fed the unsupplemented diet, vaccinated with IBD vaccine, but not challenged with IBDV strain STC (*P* < 0.05). Mean B:B ratio and BHS in Group 1 fed the diet not supplemented with AA, vaccinated with IBD vaccine, and challenged with IBDV strain STC was not significantly (*P* < 0.05) different from that of chickens in Group 5 fed the AA-supplemented diet. Chickens in Group 8 fed the diet supplemented with AA, not vaccinated with IBD vaccine, and not challenged with IBDV strain STC had significantly (*P* < 0.05) higher B:B.

TABLE 6. Mean bursal histopathological scores (BHS) and bursa to body weight (B:B) ratio of chickens with treatments of dietary supplementation with ascorbic acid, vaccination for infectious bursal disease, and challenge with infectious bursal disease virus, alone or in combination, before challenge (21 d of age) and 10 d after challenge (31 d of age) with infectious bursal virus standard challenge strain (STC)

<table>
<thead>
<tr>
<th>Group</th>
<th>AA</th>
<th>Vaccination</th>
<th>Challenge</th>
<th>Prechallenge</th>
<th>Postchallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21 d</td>
<td>31 d</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>3.49b,6</td>
<td>1.0a,6</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>4.48b</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>5.77x,7</td>
<td>1.0x,7</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>5.37b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3.45b,8</td>
<td>1.0b,8</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>6.76a</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>5.74x,9</td>
<td>1.0x,9</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>6.62a</td>
<td></td>
</tr>
</tbody>
</table>

*Means in a column with no common superscript differ significantly (*P* < 0.05).

1AA = Supplementation of diet with ascorbic acid at 1,000 ppm from 1 to 31 d of age.
2Vaccination with infectious bursal disease vaccine at 7 d of age.
3Challenge with infectious bursal disease virus strain STC at 21 d of age.
4Bursa weight divided by body weight × 1,000.
5Bursal histopathological lesions scored from 1 to 4 based on increasing severity.
6Mean B:B and BHS for chickens in Groups 1 and 2 at 21 d of age before challenge.
7Mean B:B and BHS for chickens in Groups 3 and 4 at 21 d of age before challenge.
8Mean B:B and BHS for chickens in Groups 5 and 6 at 21 d of age before challenge.
9Mean B:B and BHS for chickens in Groups 7 and 8 at 21 d of age before challenge.
Sterone levels were measured throughout the 31 d of the different treatments at the time intervals when corticosterone levels in chickens with inconsistent effect on corticosterone levels in chickens with 1989; Kutlu and Forbes, 1993), supplementation of AA with dietary supplementation of AA (Satterlee et al., 1986; Tuekam et al., 1994) that inclusion of AA at 1,000 ppm elevated serum AA levels and produced beneficial effects such as increased body weight gains, increased antibody production, and decreased mortality by providing the required AA levels not sufficiently synthesized in vivo. Chickens in Group 3 fed the diet not supplemented with AA, not vaccinated with IBD vaccine, but challenged with IBDV strain STC had the lowest serum AA levels. This finding was compatible with those of other researchers that showed that chickens infected with various infectious agents had lower serum AA levels as compared to those of controls (Squibb et al., 1955). Even though usage of various doses of viruses have been reported in challenge studies (Ismail and Saif, 1991; Van den Berg et al., 1991), the $10^{3.2}$ 50% embryo lethal dose used in the present study was able to cause clinical signs and 30% mortality to chickens in Group 3. However, no clinical signs and mortality were noted in chickens in Group 7 fed the diet supplemented with AA, not vaccinated with IBDV vaccine, but challenged with IBDV strain STC. Therefore, the supplementation of AA seems to play a role in preventing the occurrence of morbidity and mortality caused by IBDV.

Although elevated serum corticosterone levels during a variety of stress conditions has been shown to decrease with dietary supplementation of AA (Satterlee et al., 1989; Kutlu and Forbes, 1993), supplementation of AA at 1,000 ppm in the present study was found to have no consistent effect on corticosterone levels in chickens with different treatments at the time intervals when corticosterone levels were measured throughout the 31 d of the experiment. However, a decrease in corticosterone level with age was observed. This result corresponded with findings published previously (Gross, 1988; McKee and Harrison, 1995). Nevertheless, it was necessary to monitor serum corticosterone levels during the study because increased amounts of corticosterone have been implicated in reduced growth rate, decreased number of lymphocytes, reduced size of lymphoid organs, and decreased antibody response (Gross et al., 1980).

Significantly ($P < 0.05$) higher ELISA titers to IBDV were observed in chickens (Groups 5 and 6) fed the diet supplemented with AA as compared to those in chickens (Groups 1 and 2) fed the diet not supplemented with AA at 14 d after vaccination with IBD vaccine. This finding was compatible with those of previous reports that dietary supplementation of AA at 1,000 ppm increased antibody response of chickens to sheep red blood cells that were suppressed by heat stress (Pardue et al., 1985) and chickens supplemented with AA at 500 ppm had increased antibody titers to infectious bronchitis virus vaccine (Tuekam et al., 1994). Chickens (Group 7) fed a diet supplemented with AA and not vaccinated against IBDV did not have high ELISA titers to IBDV; however, they did not exhibit any clinical signs or mortality when challenged with IBDV strain STC. The suppression of morbidity and mortality in the AA-supplemented chickens may have been due to the antioxidant property of AA in that AA was able to protect immature lymphocytes from damage by free radicals due to oxidation, thus enhancing the immune response.

Ascorbic acid is involved in growth by promoting collagen synthesis, calcium and vitamin D3 metabolism, carnitine synthesis for oxidation of fatty acids, oxidation of amino acids, electron transport in the cells, and scavenging of free radicals (Combs, 1992). The present study indicates that chickens fed a diet supplemented with AA had higher body weight gains as compared to those fed a diet not supplemented with AA. This finding is compatible with a previous report that chickens benefited from dietary supplementation of AA and gained weight faster than controls (Schildknecht et al., 1986). However, no significant differences in body weight gains were observed between chickens fed an AA-supplemented diet and those fed a diet not supplemented with AA at 14 d after vaccination (21 d old). The reason for this finding is unclear. However, kidneys, which are the principal organs for chickens to synthesize AA, cannot synthesize adequate amounts of AA until after 15 d of age (Puls, 1994). Therefore, kidneys of chickens at 21 d of age are functionally and morphologically competent to synthesize sufficient amounts of AA to supply the tissues to compensate any adverse effect on growth that otherwise might have been caused by vaccination with IBD.

Lower B: B ratios were noted in vaccinated chickens compared to those in their nonvaccinated counterparts before challenge. However, no difference in BHS was seen. The finding of a lower B: B ratio due to IBD vaccination, which protects chickens from having clinical signs
and mortality, was compatible with a previous report (Van den Berg and Meulemans, 1991). In the present study, lower B:B ratios were observed in vaccinated chickens that had smaller but histopathologically unremarkable bursae as compared to the nonvaccinated counterparts. Supplementation of AA in the diet alone did not cause bursal damage in chickens when compared to vaccination with IBD vaccine alone. Therefore, supplementation of AA did not promote pathological changes in the bursa of Fabricius and did not have adverse effect on growth. In spite of lowest B:B ratios and highest BHS in chickens challenged with IBDV, chickens fed a diet supplemented with AA at 1,000 ppm did not develop clinical signs. This result may be due to the ability of AA supplementation to stimulate interferon activity by increasing the amount of interferon messenger RNA and increasing antiviral activity of interferon through the action of cyclic adenosine monophosphate (Siegel, 1975). Another possibility is that AA supplementation may speed up differentiation of lymphoid organs by increasing the activity of hexose monophosphate pathway, thus increasing circulating antibody (Dieter and Breitenbach, 1971). In addition, AA supplementation may have interfered with apoptotic or necrotic process of bursal lymphocytes during infection of IBDV. Because the use of IBD vaccine can cause a varying degree of bursal lymphocytolysis, as observed in chickens (Group 2) in the present study (Van den Berg et al., 1991), dietary supplementation of AA may alleviate the adverse effect of vaccination on chickens (Group 6). The higher B:B ratios and lowest BHS observed in chickens fed the diet supplemented with AA and vaccinated with IBD vaccine as compared to those of the AA-unsupplemented counterparts may be due to the ability of AA to protect the bursa of Fabricius from being damaged by IBD vaccine virus.

Dietary ascorbic acid supplementation may be beneficial to chickens during IBD vaccination and also during natural infections in the field. Dietary supplementation may improve body weight gain and antibody response to IBD vaccination. In chickens exposed to natural infections, ascorbic acid may prevent development of clinical signs and reduce mortality.

REFERENCES


