Ascorbic acid supplementation improved resistance to infectious bursal disease virus in chickens

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Abstract

Infectious bursal disease virus is an important immunosuppressive virus of chickens. This study was done to determine if supplementation of ascorbic acid to the diet would have a beneficial effect for protection against infectious bursal disease virus infection in chickens. Ascorbic acid (AA) supplementation at 500mg/kg in the diet decreased the mortality rate from 33.3% in infected-non-treated chickens to 13.3% in infected-treated ones. The histopathological study showed that the lesions in bursa of the treated chicken is little than that of non treated ones. AA supplementation increased the body weight gain, serum total protein, serum albumin, serum globulin and Tri-iodothyronine (T3).

Introduction

Infectious bursal disease (IBD) is widespread in chickens and of great economic importance. Most susceptible are specific- pathogen-free chickens between 3 and 6 weeks of age (11), in which mortality may reach as high as 100%. Infection during the first week after hatching may lead to severe defects of humoral immune response as a consequence of the early destruction of the bursa of Fabricius (BF). In contrast, older chickens with regressed bursa do not show signs of illness upon infection (9, 17). The acute phase of the disease lasts for about 7-10 days. Within this phase, bursal follicles are depleted of B cells and the bursa becomes atrophic. The characteristic lesions of IBD are necrosis of the BF with subsequent inflammation and atrophy of that organ. Abundant viral antigen can be detected in the bursal follicles and other peripheral lymphoid organs such as the cecal tonsils and spleen (9, 17).

Ascorbic acid (AA), the major water-soluble antioxidant, is beneficial in reducing oxidative stress (3). AA helps in protection against many viruses infection (6, 15). Supplementation of AA to the diet has a beneficial effect for protection against IBDV infection (2, 18). Ascorbic acid-supplemented chickens challenged with IBDV do not show any clinical signs or mortality (2). Dietary supplementation of AA

ameliorate the immunosuppression caused by IBDV vaccination and improve humoral and cellular immune responses (18).

Infectious bursal disease is first recorded in Egypt by El-Sergany et al. (8). IBD is still a significant threat facing the Egyptian poultry industry. Outbreak continues to occur in many flocks applying different vaccination programs with intermediate and/or oil-adjuvant vaccines. The objective of this study is to study the effects of ascorbic acid on the immune response and protection of chicken against challenge with vvIBDV.

Materials and methods

Chickens. Cup broiler chickens, obtained as one day old were used in this study. They were kept on a wire net floor, maintained on a balanced ration, obtained from the General poultry company, Egypt. No drugs or vaccines were given to the chickens along the course of experiment except those under investigation.

Ascorbic acid. AA was commercially purchased from Memphis Company.

Experiments. Two experiments were carried out;

The first experiment was conducted to study the effect of AA on protection rate against mortality among chicken challenged with very virulent (vv) IBDV, as well as improvement of immune response against virus. Chickens were divided into four groups and reared under similar conditions. First group was fed a diet supplemented with AA and does not infected with the virus. The second group was supplemented with AA and infected with the virus at 14 days of age. The third group was infected with the virus without AA supplementation. The fourth, control group was fed an identical diet, neither supplemented nor infected. The dose of AA supplementation was 500 mg/ kg ration and started at 7^{th} day old and continued till the end of the experiment. Chickens were infected with a vv IBDV, identified and titrated to contain 4x10(5) in 0.05 ml, by intramuscular injection at 14 days old. Mortality rates were recorded for 15 days post challenge. At 5, 10 and 15 days of challenge, five birds were randomly selected from each group, slaughtered and lymphoid organs (thyroid gland, spleen and BF) were dissected out and were grossly examined and specimens from BF from each subgroup were taken and fixed in 10% buffered formaline, embeded in paraffin, sectioned at 5 microns, stained with Haematoxylin and Eosin (13), and examined microscopically.

The second experiment was done to evaluate the effect of different doses of ascorbic acid on the protective effect of IBD vaccine. Forty chichens were divided into 4 groups of 10 chicks each. The first group was supplemented with 0.5 gm/ kg diet. The second group was supplemented with 1 gm / kg diet. The third group was supplemented with 2 gm / kg diet. The fourth group was left as non-supplemented vaccinated control group. The three supplemented groups were treated at 7 days of age till the end of experiment. All birds were vaccinated at day 14with IBD vaccine (Ivaz, Italy). At the end of the experiment, 21 days old, body weight gain was determined**.** Blood samples were obtained and serum was separated for determination of total serum protein, albumin, globulin, and T3.

Chemical tests. Serum sample was used for measurement of total protein according to Cornall et al. (5) and albumin according to Doumas (7).

ELISA test: Serum samples were assayed for T3 using commercial ELISA system test kits (Human, Germany) according to Chopra (4)**.** The test was done according to the directions supplied by the manufacturer.

Results

First experiment:

Clinical and postmortem findings. Groups 1(supplemented-non infected) and group 4 (non supplemented-non infected) did not show any disease or mortalities. Infected-non supplemented chickens (group2) developed a fatal disease. The first clinical symptoms appeared in the form of ruffled feathers and white or watery diarrhea. Later, these signs were followed by anorexia, depression, trembling, and severe prostration. 33.3 % of chickens died between 3 and 15 days post infection, showing typical gross lesions of IBD. Group 3, that had AA supplementation and infected with the virus, showed 13.3 % mortalities. The gross lesion encountered in postmortem examination was moderate and less severe than that of group 2.

Pathological lesions. The control group, non infected and non supplemented chickens (group4), showed normal lymphoid follicles in bursa (fig. 1). The infected non supplemented group (group3) showed severe pathological lesions. There were areas of discrete lymphoid necrosis with severe lymphoid depletion and hemorrhages among lymphoid follicles, and other follicles showed edema in between the follicles (Fig.2). Bursa from supplement and infected chicken (group2) showed mild focal

necrosis of the lymphoid follicle after challenge with IBD virus (Fig.3). AA supplemented non infected chicken (group1) showed lymphoid hyperplasia in the bursa (Fig.4).

Second experiment.

Chemical finding. As shown in Table 1, AA supplementation markedly increased the serum total protein, albumin, globulin, T3 and the body weight gain. It has been noticed that the effects were appropriated to the doses of AA. The improvement in the body weight gain, serum total protein, albumin, globulin and T3 increased with the dose of AA. However, the noticeable improvement was seen when the dose increased from 0.5 gm to 1 gm, while mild improvement was noticed in between 1 gm and 2 gm treatment.

Table 1.Effect of ascorbic acid administration in different doses on total protein, albumin, globulin, T3, and body weight gain in-21day-old chicks

Parameter	**Control**	**0.5gm AA**	**1gm AA**	**2gm AA**
T.protein (g/dl)	2.95	2.99	3.89	4.30
Albumin (g/dl)	1.26	1.28	1.48	1.77
Globulin (g/dl)	1.68	1.71	2.41	2.53
T3 (ng/ml)	0.90	1.20	1.50	1.70
Body weight (g)	800	820	860	880

Fig.1. Bursa from control non-infected, non supplemented chicken, notice normal lymphoid follicles. Haematoxylin &Eosin· 100.

Fig.2.Bursa from infected chicken with IBDV showing atrophy of lymphoid follicles, fibroplasias and multifocal necrosis in the lymphoid follicles. Haematoxylin & Eosin.100.

Fig.3. Bursa from ascorbic acid supplemented chicken challenged with IBDV. Notice mild focal necrosis of the lymphoid follicles. Haematoxylin & Eosin. 100.

Fig.4. Bursa from ascorbic acid-treated chicken. Notice lymphoid hyperplasia of the lymphoid follicles. Haematoxylin & Eosin. 100.

Discussion

The IBDV is caused by a double-stranded and segmented ribonucleic acid virus, family birnaviridae. Clinical and subclinical infection with IBDV may cause immunosuppression. The replicating virus can have both direct and indirect effects on the cells of the immune system (14). Inhibition of the humoral immunity is attributed to the destruction of immunoglobulin-producing cells by the virus. Other mechanisms such as altered antigen-presenting and helper T cell functions may also be involved (17). Stimulation of the defense mechanism is of great importance for prevention of all sorts of immunosuppressive influences in poultry breeding. The aim of this work was to investigate the effect of AA supplementation on the protection against IBDV.

The first approach was to identify the role of AA supplementation in protection against mortalities of chickens after challenge with IBDV. AA administration resulted in high protection rate against mortality among chicken challenged with vvIBDV. It decreased the mortalities from 33.3% in non treated group to 13.3 % in treated one. Results of microscopical examination of bursae support our findings as lymphoid hyperplasia was found in ascorbic acid treated chicken. Also, the lymphoid follicle of BF showed mild lesion in supplemented-infected group (fig 3) compared with infected non-supplemented group (fig 2). These results are in agreement with that of Amakye-Anim et. al., (2) who mentioned that AA-supplemented chickens challenged with IBDV did not show any clinical signs or mortality. They also mentioned that higher ELISA titers to IBDV were observed in vaccinated chickens supplemented with AA as compared to AA-unsupplemented counterparts. Wu et. al., (18) also mentioned that the number of CD8 (+) in spleen and IgM(+) cells in bursa were significantly higher in AA supplemented chickens. The number of anti-IBDV IgG antibody secreting cells in spleen was significantly higher in AA supplemented group.

The second approach was to study the effect of different doses of AA supplementation on the serum total protein, albumin, globulin, T3 and body weight gain. The results showed AA supplementation significantly increased the total protein, albumin, globulin, T3, and the body weight gain in 21-day-old chickens. The increases were seen with the different doses of AA. However, it was very clear when the dose increased from 0.5 gm to 1 gm AA. In fact, similar results were seen when the chicken supplemented by thymus extract as immunostimulant. Oral administration

of thymus extract (1 m l/kg) increased the total protein, albumin, globulin, T3, T4 and the body weight gain in 21-day-old chick. In addition, it increased the total lymphocytic count (1). Also, their results showed a marked increase in antibody titers in thymus treated chickens (1). Addition of 5% freeze-dried IBD virus (IBDV)-immune bovine colostrum to the diet of chickens prevented infection when housed in an IBDV-contaminated environment (12).

How does ascorbic acid help in protection against IBDV infection? Firstly, the marked elevation observed in the level of T3 could explain the activation of the immune system. Rober et al. (15**)** and Kaneko et al. (10) found an inverse relationship between hyperthyroidism and hypocorticocism and that the immunosuppressive action of corticosteroid is reversed by hyperthyrodism. Secondly, infection with IBDV stimulates macrophages to produce nitric oxide (NO) and other cytokines with anti-proliferative activity (17). Short-term incubation of IBDV with heterophils causes an activation of cellular oxidative burst (11). Oxidative stress is a reflection of excess intracellular concentrations of reactive oxygen species and one of the important indicators of cellular damage. Oxidative stress may interfere with the immunological mechanisms involved in viral clearance, thus facilitating viral replication and enhancing cells and tissue damage (15). Antioxidants, such as ascorbic acid transforms free radicals into less reactive species, thereby limiting their toxic effects.

In conclusion, this work shows the importance of stimulation of the defense mechanism for prevention of immunosuppressive influences in poultry breeding. AA supplementation greatly improves the protection against IBDV.

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