Effect Of Ascorbic Acid Supplementation On Improvement The Resistance To Infectious Bursal Disease Virus In Chicken

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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 diet decreased the mortality rate from 33.3% in infected-non-treated chickens to 13.3% in infected-treated ones. The gross lesions and histopathological results showed that the lesions in bursa of the treated chicken are less 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 of great economic importance in chickens. Most susceptible are specific- pathogen-free chickens between 3 and 6 weeks of age *(1),* in which mortality may reach as high as 100%. Infection during the first week after hatching may lead to severe suppression of humoral immune response as a consequence of the early damage of the bursa of Fabricius (BF). In contrast, older chickens with regressed bursa do not show signs of illness upon infection *(2, 3)*. 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. Abundant viral antigen can be detected in the bursal follicles and other peripheral lymphoid organs such as the cecal tonsils and spleen *(2, 3)*.

Ascorbic acid (AA), the major water-soluble antioxidant, is beneficial in reducing oxidative stress *(4)*. AA helps in protection against many viruses infection *(5,6).* Supplementation of AA to the diet has a beneficial effect for protection against IBDV infection *(7,8).* Ascorbic acid-supplemented chickens challenged with IBDV do not show any clinical signs or mortality *(7).* Dietary supplementation of AA ameliorate the immunosuppression caused by IBDV vaccination and improve humoral and cellular immune responses *(8).*

Infectious bursal disease is first recorded in Egypt by ***El-Sergany et al. (9).*** 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 was to study the effects of ascorbic acid on the immune response and protection of chicken against challenge with VVIBDV.

MATERIALS AND METHODS

Chickens

Eighty SPF chicks obtained as SPF embryonated chicken eggs from Koam Osheim, El-fayoum governorate and hatched in our laboratory, were used in this study. No drugs or vaccines were given to the chickens along the course of experiment except those under investigation.

IBDV

IBDV was obtained from Vet. Serum and Vaccine Research Institute Elabasya.

Feed

Feed was obtained from Cairo Poultry Company.

Ascorbic acid

Ascorbic acid was commercially purchased from Memphis Company Egypt .

IBD vaccine (Ivaz, Italy).

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 VVIBDV, as well as improvement of the immune response against IBDV. Forty chickens were divided into four equal groups and reared under similar conditions. First group was fed a diet supplemented with AA and did not infected with the virus. The second group was supplemented with AA and infected with the UIBDV at 14 days of age. The third group was infected with the virus without AA supplementation. The fourth, the control group was fed normal balanced diet and neither supplemented nor infected. The dose of AA supplementation was 500 mg/ kg ration and started at 7th day old and continued till the end of the experiment. Chickens were infected with VVIBDV, 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 P.M. examination was carried out, and specimens from BF were taken and fixed in 10% buffered formaline, embeded in paraffin, sectioned at 5 microns, stained with Haematoxylin and Eosin ***(10),*** 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 four equal groups. 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 kept as non-supplemented vaccinated control group. The three supplemented groups were treated at 7th of age till the end of experiment, 29 days old. All birds were vaccinated at day 14with IBD vaccine (Ivaz, Italy). At the end of the experiment, body weight gain was determined. Blood samples were obtained and sera were separated for determination of total protein, albumin, globulin, and T3.

Chemical tests

Serum sample was used for measurement of total protein ***(11)*** and albumin ***(12).***

ELISA test: Serum samples were assayed for T3 using commercial ELISA system test kits (Human, Germany) ***(13).*** The test was done according to the directions supplied by the manufacturer.

RESULTS

First experiment

Clinical and postmortem findings

Group 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, hemorrhagic diarrhea and severe prostration. 33.3 % of chickens died between 3 and 15 days post infection, firstly the bursa was oedematus containing a gelatineous transudate covering 'the serosal surface and longitudinal striation on the surface became prominent and later it became hemorrhagic (Fig. 2). Moreover, hemorrhage on the leg muscle was recorded (Fig.1). On the other hand, increased mucous in intestine was observed. Group 3, that had AA supplementation and infected with the virus, showed 13.3 % mortalities. The gross lesions encountered in postmortem examination were moderate and less severe than that of group 2.

Pathological lesions

The control group, non infected and non supplemented chickens (group 4), showed normal lymphoid follicles in the bursa (Fig.3). 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, other follicles showed edema in between the follicles (Fig.4). Bursa from supplemented and infected chicken (group2) showed mild focal necrosis of the lymphoid follicle after challenge with IBD virus (Fig.5). AA supplemented non infected chicken (group1) showed lymphoid hyperplasia in the bursa (Fig.6).

Second experiment

Biochemical 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 at dose of 1 and 2 gm treatment.

Table 1. Effect of ascorbic acid administration with different doses on total protein, albumin, globulin, T3, and body weight gain at 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. Hemorrhages of the leg muscle typical in IBD

Figure 2. Edematous and hemorrhagic cloacal bursa(white arrow)in infected non treated group and bursa has a gelatinous yellowish transudate covering the serosal surface(black arrow)

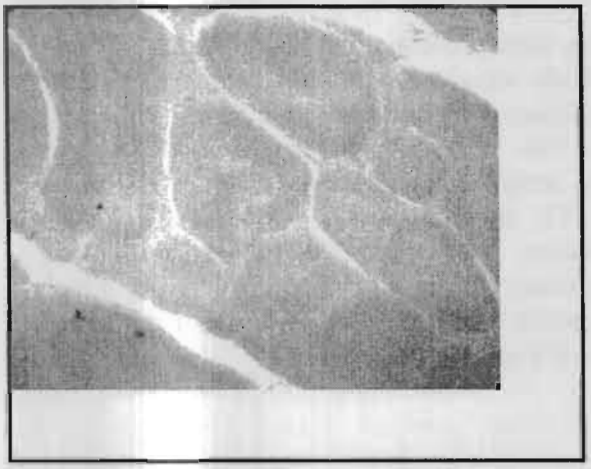

Fig.3. Bursa from control (non-infected, none supplemented) chicken, notice normal lymphoid follicles. Haematoxylin &Eosin X50.

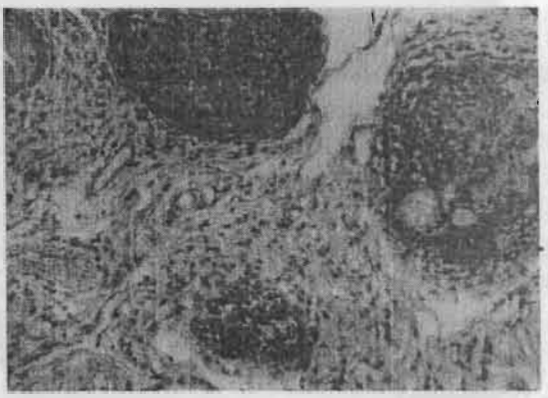

Fig.4. Bursa from infected chicken with IBDV showing atrophy of lymphoid follicles, fibroplasia and multifocal necrosis in the lymphoid follicles. Haematoxylin & Eosin.X 80.

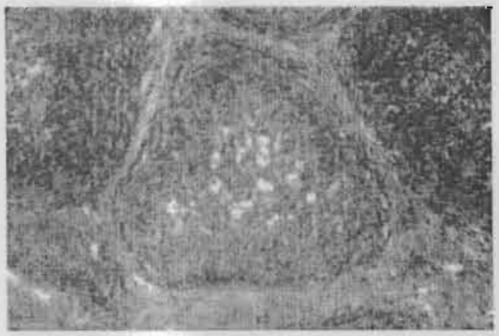

Fig.5. Bursa from ascorbic acid supplemented chicken challenged with IBDV. Notice mild focal necrosis of the lymphoid follicles. Haematoxylin & Eosin.X80.

Fig.6. Bursa from ascorbic acid-treated chicken. Notice lymphoid hyperplasia of the lymphoid follicles. Haematoxylin & Eosin. X50

DISCUSSION

The IBDV is caused by a double-stranded and segmented genome. 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 ***(3).*** Stimulation of the defense mechanism is of great importance for the prevention of all sorts of immunosuppressive influences in poultry breeding.

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 (86.7%) against mortality among chicken challenged with VVIBDV, decreased mortality 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. 5) compared with infected non-supplemented group (Fig 4). The presented results are consistent with that of **Amakye-Anim et. al.,** ***(7)*** 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. The number of CD8 (+) in spleen and IgM(+) cells in bursa were significantly higher in AA supplemented chickens ***(8)***. The number of anti-IBDV IgG antibody secreting cells in spleen was significantly higher in AA supplemented group.

On the other hand significant increase in the serum total protein, albumin, globulin, T3 and body weight gain in chickens was associated with supplemented with AA the increase was very clear when the dose was increased from 0.5 gm to 1.0 gm/kg diet.

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. An inverse relationship between hyperthyroidism and hypocorticocism and that the immunosuppressive action of corticosteroid is reversed by hyperthyrodism ***(15, 16)***. Secondly, infection with IBDV stimulates macrophages to produce nitric oxide (NO) and other cytokines with anti-proliferative activity ***(3).*** Short-term incubation of IBDV with heterophils causes an activation of cellular oxidative burst ***(1).*** Oxidative stress is a reflection of excess intracellular concentrations of reactive oxygen species (ROS) which is one of the important indicators of cellular damage. While the use of AA decreases cellular damage with mild focal necrosis of lymphoid follicle as shown in Fig. 5, 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 ***(4).***

In conclusion, the current study showed that, ascorbic acid can improve the body weight and increases the defense mechanism of chicken and reduces immunosuppression in poultry breeding. Also, AA supplementation greatly improves the protection against IBDV and increasing the efficacy of IBD vaccine.

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الملخص العربى

تاثير اضافة حامض الاسكوربيك على تحسين مقاومة الدجاج لمرض الجمبورو

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يعد مرض الجمبورو من اهم الامراض الفيروسية التى تحدث تثبيط للجهاز المناعى فى الدجاج و لذلك فقد قمنا بعمل دراسة لاثبات هل إضافة حامض الاسكوربيك لعليقة الدجاج لها تاثير نافع فى الحماية من الإصابة بمرض الجمبورو. و قد اثبتت التجربة ان إضافة حامض الاسكوربيك بتركيز ٥٠٠ مج لكل كج من عليقة الدواجن يخفض نسبة الوفيات من ٣٣٣% فى المجموعة الغير معالجة الى ٣و١٣ فى المجموعة المعالجة بحامض الاسكوربيك . و لقد دعمت هذه النتيجة الدراسات التشريحية و الباثولوجية التى اظهرت و جود تغيرات بسيطه فى المجموعة المعالجة مقارنة بالغير معالجة و علاوة على ذلك فقد و جد ان اضافة حامض الاسكوربك يزيد الوزن و البروتين الكلى لمصل الدم و التراى ايودو ثيرونين.