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# EFFECT OF ASCORBIC ACID ON BROILER CHICKS INFECTED WITH VIRULENT INFECTIOUS BURSAL DISEASE VIRUS

(With 3 Fig. & 2 Tables)

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# تأثير حمض الاسكوربيك / فيتامين ج ا على عدوى فيروس الجمبورو الشديد الضراوه فى بدارى الكتاكيت

كمال الزناتى

تم دراسة تأثير التركيزات المختلفه من حمض الاسكوربيك المضاف فى العليقه من الاسبوع الاول من عمر مجموعات كتاكيت الدجاج حتى نهاية التجربه على العدوى بفيروس الجمبورو الشديد الضراوه والذى تم اعطاؤه عن طريق العين عند عمر أربع أسابيع لمجموعات الكتاكيت .

أوضحت النتائج ان مجموعات الكتاكيت التى أضيف لها فيتامين ج فى العليقه أظهرت أعراض أكلينيكيه وآفات تشريحيه فى كيس غدة فبريشيس ومناطق أخرى وكذلك نسب الاصابه والوفيات أقل من مجموعة الكتاكيت التى لم يضاف لها فيتامين ج .

فى اختبار الترسيب فى الآجار كانت نسب غدد الفبريشيس الموجبه أعلى فى مجموعة الكتاكيت التى لم يضاف لها فيتامين ج وأقل فى المجموعات التى تم أضافة فيتامين ج لها .

تبين أن تأثير فيتامين ج فى العليقه ضد عدوى فيروس الجمبورو تعتمد على الكميه المضافه من الفيتامين وأن أحسن النتائج كانت عند تركيز ٣٣٠ مجم فيتامين ج لكل كيلو جرام عليقه .

تم أستنتاج أن أضافة فيتامين ج على العليقه يزيد من مقاومة بدارى الكتاكيت ضد فيروس الجمبورو الشديد الضراوه .

## SUMMARY

The effect of virulent infectious bursal disease (IBD) virus on broiler chick groups fed at one week of age diets containing different concentration of ascorbic acid (AA) (group I, 0 mg AA/kg, group II, 220mg AA kg; group III, 330 mg AA/kg and group IV, 660 mg AA/kg diet, respectively) was studied. At four weeks of age, broiler chicks in different groups were infected intraocularly with $10^{5.7}$ $EID_{50}$/0.1 ml virulent IBD virus. The severity of clinical manifestations, bursal lesions and other IBD pathological lesions were reduced in AA diet supplemented group chicks (group II, III IV). Morbidity and mortality rates were less in AA receiving group chicks in comparison with group chicks supplemented no AA. Positive ground bursae in agar gel precipitation test from infected IBD-group chicks were high (66.67%) in chicks receiving no AA(group I), while low (40.00, 28.33 and 45.00%) in other AA supplemented diet group chicks (group II, III, IV) respectively. The effect of AA on IBD-infection was dose dependent. The best results were obtained at the level of 330 mg AA/kg diet (group III). It was concluded that AA increase the resistance in broiler chicks against virulent IBD-infection.


*Keywords:* Ascorbic acid chicks, Bursal disease virus.

## INTRODUCTION

Because chickens can synthesize ascorbic acid (AA), dietary supplementation normally is not required *(BELL and FREEMAN, 1971; PARDUE and THAXTON, 1986)*. Under stressful circumstances, however adequate amounts of AA may not be synthesized and supplementing the diet with AA may be benificial *(PEREK and KENDLER, 1963 and BELL and FREEMAN, 1971)*.

AA is important for the optimal functioning of the immune system. Thus, it has direct virucidal and bactericidal activity against a number of pathogens in vitro *(WHITE et al., 1986)*, it enhances the production of interferon by cells infected with NewCastle disease *(DAHL and DEGRE, 1967)*.

AA increased the resistance against infectious bronchitis virus challenge *(DAVELAAR and VAN DEN BOS, 1992)* and reduced the incidence of pericarditis or death following air sac challenge with Escherichia coli *(GROSS et al., 1988). YOTOVA et al. (1989)* found that the mortality rate due to Marek's disease was lower and the immune reactivity was higher if broilers originating from parent treated with various doses of AA.

*SATTERFIELD et al. (1940)* demonstrated that chickens infected with fowl typhoid has reduced plasma levels of AA, whereas the addition of AA reduced early but not total mortality from fowl typhoid infection. Moreover, viral infections are associated with a rapid decrease in circulating AA concentrations. *BEISEL (1982)* suggesting that whilst the AA concentration may be optimal for healthy birds, it is suboptimal during periods of infection. AA is a potential anti-immunosuppressive agent in the chickens *(PARDUE and THAXTON, 1984).*

Infectious bursal disease represents one of the most economic important contagious viral infection of young chickens inducing high morbidity and mortality as well as pathological effect on bursa Fabricius (BF) and immunosuppression, this study was undertaken to asses the effects of AA feed supplementation on the IBD in broiler chicks infected with virulent IBD virus.

## MATERIAL and METHODS

**Chicks:**

300 one-day-old broiler chicks (Arbor Acres) were purchased from Beni-Mur, governmental hatchery. The chicks were brooded in a strict isolated pen and provided at libitum commercial broiler feed and water.

**Ascorbic acid (AA):**

AA was obtained from the Roche chemical Division of Hoffman-La Roche, Inc. Nutley, New Jersey, as an ethyl cellulose-coated product that was 97.5% AA. This product was added to the basal commercial broiler feed at doses of 0 mg AA/kg, 220 mg AA/kg, 330 mg AA/kg & 660 mg AA/kg feed as mentioned below.

**Ascorbic acid diets and chicks:**

At the age of one week, the chicks were divided into four groups (I, II, III & IV) of 60 birds each, the rest of chicks were kept as non-treated non-infected control group (Fifth group) Chicks in all groups were received the same commercial

broiler feed supplemented with the following levels of AA: group I, 0 mg AA/kg feed; group II, 220 mg AA/kg feed; group III, 330 mg AA/kg; group IV, 660 mg AA/kg feed. Feed was consumed within 5 days of mixing and experimental feeds were provided to the birds till the end of the experiments.

**Embryonated chicken eggs (ECE):**

9-11-day-old ECE were supplied by Faculty Agriculture Farm, Assiut University and used for virus titration and virus reisolation.

**Agar gel precipitation (AGP) test:**

The test was carried out by the micro-method on microscope slides using 1% DIFCO-Noble agar in 8% sodium chloride solution. The antigen was prepared from twicely freezing and thawing ground bursae of IBDV-infected chickens. The test was read 24-48 h. post incubation at 3°C.

**IBD-Hyperimmune serum:**

IBD-antiserum was locally prepared in rabbits in our Poultry Diseases Department.

**Virulent IBD virus:**

Ground bursae from chickens experiencing clinical IBD ($3^{rd}$ chick passage) was prepared in our department and used in challenge test. Embryo infective dose- fifty ($EID_{50}$) was determined in ECE after Reed and Muench (1938).

**Challenge test:**

Firstly, chicks of all groups were tested before challenge for freedom of IBD-precipitating antibodies by AGP-test. AT four weeks of age, birds in group I, II, III and IV were infected intra-ocularly with $10^{5.7}$ $EID_{50}$/0.1 virulent IBD virus per bird. Chicks in the fifth group were kept as non infected control group. All birds were observed for 8 days. Clinical signs, mortalities and different postmortem lesions were recorded. Lesions in the BF were expressed as 0, normal size and white colour; +1. creamy or slight hemorrhagic and enlarged bursae; +2 double normal size or more edematous bursae with petechial or ecchymotic hemorrhages on the internal and serosal bursal surface; +3 grayish-colour atrophied bursae.

Specificity of deaths was confirmed through testing ground BF in AGP test, isolation of the virus from BF and testing their CAM in AGP-test. At the end of the experiment, the rest of chicks in different groups were killed and examined. Bursae from dead and killed chicks were test in AGP-against IBD-serum.

## RESULTS

**Clinical signs in infected IBDV broiler chicks:**

Typical IBD clinical signs (anorexia, depression and diarrhea) were more obvious 60-72 hours post infection (PI) in group chicks I but less and 12-24 hours later in the group of chicks No. II, III and IV. Morbidity rate was high (78%) in the group of chicks No. I and to some extent low (52, 36 and 44%) in group of chicks No. II, III and IV respectively. The clinical signs are followed by severe prostration and deaths. Time of death occurrence PI, daily number of dead birds and mortality percentage in all infected group chicks are shown in Table, 1. Fig.,1 illustrate number of dead birds/day PI. Chicks in non infected group V are quite healthy.

**Post-mortem findings:**

Gross lesions: By $3^{rd}$ and $4^{th}$ day PI, bursae from dead birds of the first group became creamy, congested, increased in size. On $5^{th}$ and $6^{th}$ PI, bursae were nearly double its normal size or more, edematous with petechial or ecchymotic hemorrhages on the mucosal and serosal surfaces Fig. 2. Extensive hemorrhages were also been throughout the entire bursae in the group of chicks No. I. On $7^{th}$ and $8^{th}$ day PI, bursae were atrophied and greyish in colour. High reduction of bursal lesions was noted in group of hkicks No. III in comparison with other infected groups. Other pathological lesions include hemorrhages in the thigh and pectoral muscles Fig. 3, hemorrhages on the mucosal surface of the proventriculus and at the junction of the proventriculus and gizzard in birds died on $4^{th}$ or $5^{th}$ day PI were frequently more evident in group of chicks No. I and were less in the other infected chick group (II, III, IV). Ecchymotic hemorrhages in the thigh muscles were observed in the chicks group I, II, * IV and were less commonly seen in group chicks III Fig. 3. Incidence of BF lesions in different infected groups as expressed 0, +1, +2, & +3 are seen in Table (2).

At the end of experiment, killed birds in group I, II, III & IV showed mainly pathological lesions in BF in some birds (Table, 2) and the others had no evidence of bursal damage. Group chicks V had no specific PM lesions.

**AGP-test:**

All ground bursae from dead birds were positive in AGP-test. The percentage of positive bursae in dead birds were 28.57, 12.19 8.51 and 15.38 in group I, II, III and IV, respectively; and percentage of positive bursae (from dead and

killed birds) were 66.67, 40.00, 28.33 and 45.00% in chicks groups I, II, III and IV respectively.

## DISCUSSION

Severity of clinical manifestations, pathological postmortem findings (fig. 2&3), mortality rates and number of positive IBD- infected bursae in IBD infected broiler chicks were reduced in group chicks No. II, III & IV receiving different levels of AA (220 mg AA/Kg feed, 330 mg AA/Kg feed, and 660 mg AA/Kg feed) respectively in comparison with group chicks I receiving no AA (0 mg AA/Kg feed) indicating that AA feed supplementations in broiler chicks increase the resistance against IBD infection.

Clinical signs appeared (12-24 hours) earlier in goup chicks No. I (receiving no AA) and the morbidity and mortality rates were highest in this group, while they were lowest in group chicks receiving 330 mg AA/Kg feed (Table 1). The number of affected bursae and extensive bursal damage in dead and killed chicks as shown in Table 2, which revealed that AA increase the resistance against IBD-infection. These findings agree with previous reports; which evidenced that addition of AA reduce early but not total mortality from fowl typhoid infection *(SATTERFIELD et al., 1940)*. Increased amount of AA in the feed reduced the incidence of pericarditis or deaths following challenge with Escherichia coli *(GROSS et al., 1988)*. Mortality rate due to Marek's disease was lower and immune reactivity was higher if broilers originating from parent treated with various doses of vitamin C *(YOTOVA et al., 1989)*. DAVELAAR and VAN DEN BOS (1992) reported that different levels of AA as feed supplement of broiler chicks increase the resistance against infectious bronchitis. On the other hand, *LESNIK et al. (1986)* found that administration of AA in the drinking water did not significantly affect the antibody responsse or mortality rate of chicks experimentally infected with Marek's disease, avian sarcoma or avian myeloblastosis viruses. The effect of AA on body resistance which can be determined by the severity of clinical signs, morbidity, mortality percentage as well as bursal lesions and number of positive bursae in AGP-test against IBD-infection was dose dependent. The level of 330 mg AAlKg feed was most effective in broiler chicks on resistance against IBD-infection). Similar results was previously reported by *DAVELAAR and VAN DEN BOS (1992)* who stated that the effect of AA on the resistance against infectious bronchitis was dose dependent. The present

results indicated that supplemental AA in feed of broiler chicks is beneficial to protect the chicks to some extent from the adverse effects (high morbidity and mortality and severe bursal damage) of IBD-infection. In addition, AA is accepted as a general antioxidant and is a potential anti-immunosuppressive agent in chickens (*PARDUE and THAXTON, 1984*).

From above data, it is concluded that, AA supplemented feed for broiler chicks is necessary and very useful to increase the resistance against IBD-infection.

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## LEGENDS

**Fig. 1:** Daily deaths in chick groups receiving different Ascorbic acid levels.

**Fig. 2:** a. Noral size BF (birds in group V) 33 day old chickens,

b. Congested slight hemorrhages, enlarged BF (birds in group II) Five days PI (33 day old).

c. Edematous double its normal size or more BF with hemorrhages (birds in group I) Five days PI (33 day old).

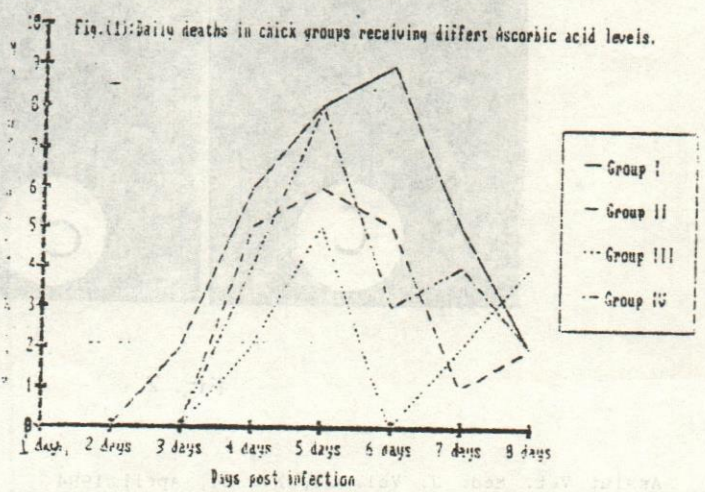
d. Atrophied, greyish BF (birds in group I) 8 days PI (36 day old).

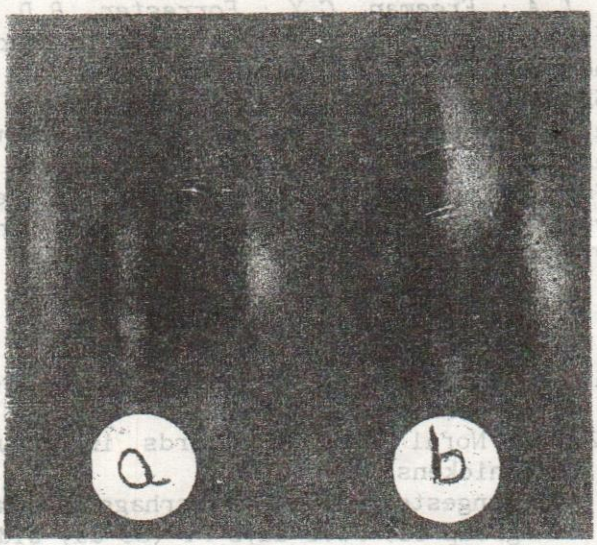
**Fig. 3:** a. Normal thigh and pectoral muscles (birds in group V) 33 day old chickens.

b. Slight congestion and hemorrhages on pectoral & thigh muscles (birds in group III) Five days PI (33 day old).

c. & d. Extensive hemorrhages on Pectoral & thigh muscles (birds in group I & II) Five days PI (33 day old).

Fig. 1





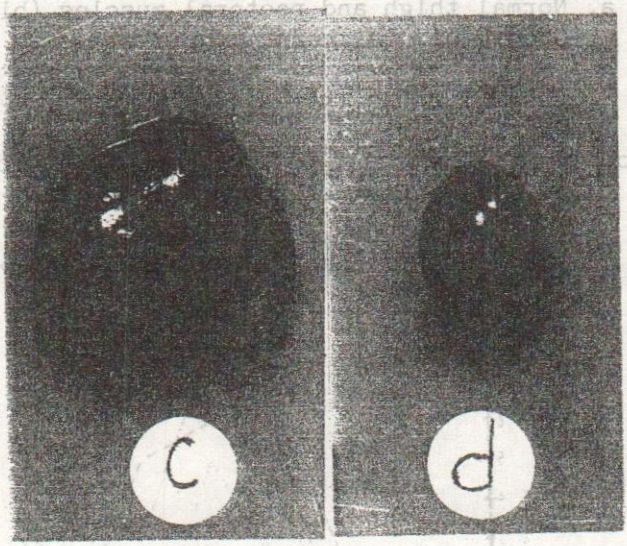




Fig. 2

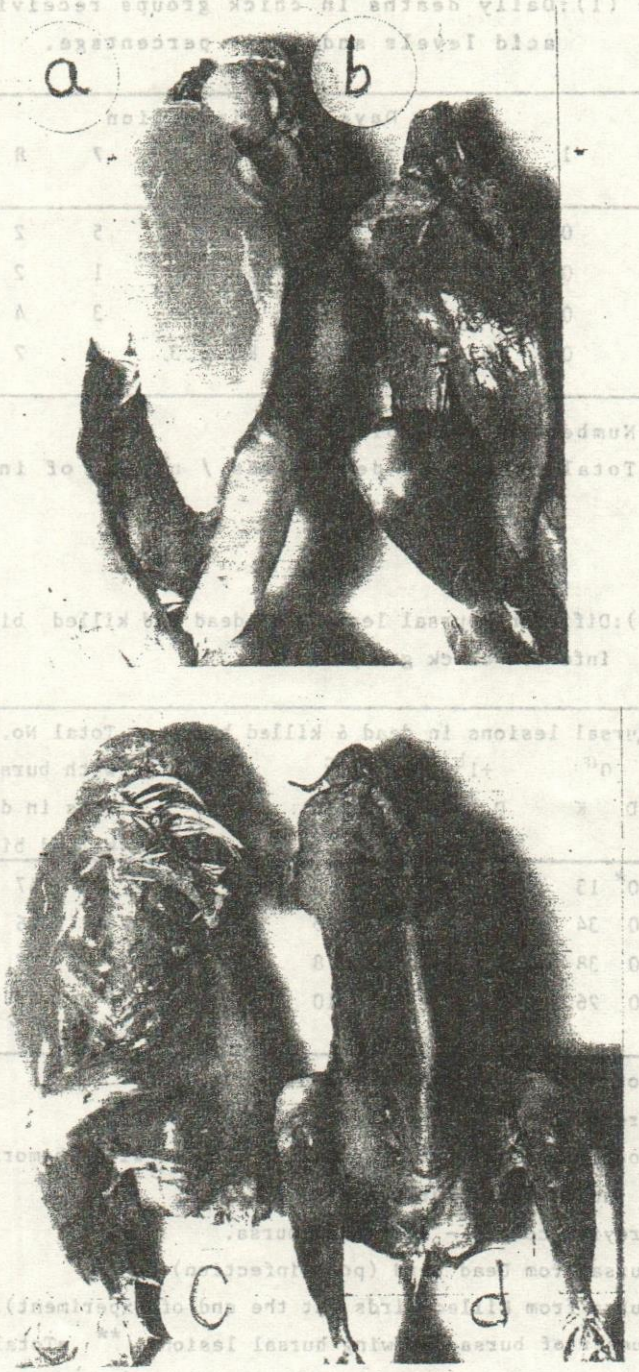



Fig. 3

Table (1):Daily deaths in chick groups receiving differt Ascorbic acid levels and their percentage.

| Group | Days post-infection 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total No. of dead birds | Mortality % |
|---|---|---|---|---|---|---|---|---|---|---|
| I | 0* | 0 | 2 | 6 | 8 | 9 | 5 | 2 | 32 | 53.30** |
| II | 0 | 0 | 0 | 5 | 6 | 5 | 1 | 2 | 19 | 31.70 |
| III | 0 | 0 | 0 | 2 | 5 | 0 | 3 | 4 | 13 | 21.70 |
| IV | 0 | 0 | 0 | 4 | 8 | 3 | 4 | 2 | 21 | 35.00 |

* = Number of dead birds.

** = Total number of dead birds / number of infected birds.

Table (2):Different bursal lesions in dead and killed birds of different infected chick groups.

| Group | Bursal lesions in dead & killed birds 0[a] D | 0[a] K | +1[b] D | +1[b] K | +2[c] D | +2[c] K | +3[d] D | +3[d] K | Total No.of bursae with bursal lesions in dead & killed birds | Bursal lesion % ** |
|---|---|---|---|---|---|---|---|---|---|---|
| I | 0* | 13 | 4 | 0 | 21 | 9 | 7 | 6 | 47 | 78.30 |
| II | 0 | 34 | 1 | 0 | 51 | 5 | 3 | 2 | 26 | 43.30 |
| III | 0 | 38 | 3 | 0 | 9 | 8 | 1 | 1 | 27 | 36.70 |
| IV | 0 | 26 | 0 | 0 | 16 | 10 | 5 | 3 | 47 | 56.70 |

0[a] = Normal size and white colour bursa.

+1[b] = Creamy or slight hemorrhagic enlarged bursa.

+2[c] = double normal size edematous bursa with hemorrhages on internal and serosal surface.

+3[d] = Greyish colour - atrophied bursa.

D = Bursa from Dead bird (post infection).

K = Bursa from Killed birds (at the end of experiment).

* = Number of bursae showing bursal lesions. ** =Total number of bursae with lesions in dead and killed birds/number of infected birds.