

## INFLUENCE OF AMMONIA AND NEWCASTLE DISEASE ON THE BROILER PERFORMANCE AND THE ROLE OF ASCORBIC ACID IN RETARDING THE STRESS EFFECTS

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### SUMMARY

The effect of multiple stresses on chick performance is of interest. In this work the effect of ammonia and NDV stresses on broiler performance and the role of ascorbic acid in the alleviation of these effects were studied. The results indicated that:

1. Exposure of broilers to ammonia stress early in life affected organ's weight in various ways i.e. decreased than the control then increased with age and ascorbic acid supplementation.
2. NDV challenge resulted in more organ's weight as a single stress than with ammonia and ascorbic acid supplement.
3. Ascorbic acid retarded the stressfulness effect on bird's internal organs.
4. Haematological changes were obvious in single stress or double without ascorbic acid treatment. Ammonia increased Heterophil/lymphocyte (H/L) ratio in early life then declined with age and ascorbic acid supplement.
5. Exposure of birds to each stress decreased food/gain ratio but the supplementation with ascorbic acid improved this ratio and mortality rate.
6. The ammonia as a stress factor affected the liver and kidneys with necrotic changes, and

the immune organs (thymus, spleen and bursa of Fabricius) with lymphocytic depletion. Such lesions appeared more extended and severe on using NDV instead of ammonia. Ascorbic acid treated ammonia alone or with NDV stress got more improvement in the degree of the previously mentioned lesions with hyperplasia of reticular cells in the lymphoid organs.

### INTRODUCTION

Several studies on animal and poultry showed that, stressors in their environment halts their normal performance (Gross and Siegel, 1983; Arjona et al., 1988 and MacFarlane and Curties, 1989).

Exposure of broilers to ammonia reduces their feed efficiency and results in deciliation of upper portion of trachea, degeneration of liver and congested firm lungs (Caveny and Quarlies, 1973 and Rodney et al., 1991).

The complex nature of ND infection which depends on immunization state, virulence of NDV and hygienic conditions, affect broiler performance seriously (El-Sadawi et al., 1984; Gross, 1984; and Dowidar et al., 1986).

It has been proved that ascorbic acid retard the stressor effects. It has a direct growth enhancement on, gain/food ratio, weight of bursa of Fabricius, thymus and has viricidal effect against a number of pathogens (Davelaar and



VanDen Bos, 1972; Dahl and Degre, 1986; and Takahashi et al., 1991.

The purpose of the present work is to evaluate the effects of ammonia stress and the NDV challenge. Also to evaluate the role of ascorbic acid (A.A) in alleviating the bird stress-response and consequent performance through determination of:

- A- Organ's weight (liver, spleen, thymus, bursa of Fabricius, Kidney and heart) as indirect measure of stress (Rodney et al. 1991).
- B- Food intake, body weight gain, and food/gain ratio.
- C- Mortality rates (post NDV challenge).
- D- Haematological changes with regard to Heterophil/Lymphocyte ratio and serum protein.
- E- Histopathological changes in internal organs, kidney, liver, heart, thymus, spleen and bursa of fabricius.

## MATERIAL AND METHODS

- 1- Chickens: One hundred, day-old Lohman chicks allocated into 4 separate groups (4 separate rooms each of 25) at the Department of Poultry Diseases, Fac. Vet. Med., Cairo University.
- 2- They were brooded and reared on deep litter floor (straw), lights allowed 24 hours with yellow bulbs. Feed and water allowed ad-libitum. Ventillation and celling for ammonia gas was controlled through the window and the door.
- 3- Ammonia stress: Was done for groups 3 and 4 at the age of 7 days by exposing the chicks to 100 ppm ammonia till the age of 35 day. The liberated ammonia given off through dissolving concentrated ammonia solution (30%). Daily check and supplement was done using Katagava gas analyser with ammonia-tube indicator.

4- Newcastle disease virus: Challenge was done with field isolate propagated on ECE, 1 ml  $10^6$  at the age of 35 days intramuscularly for groups 2 and 4.

5- Ascorbic acid: Rovimix-C, Hofman la-Roche Inc. given at 300 mg/kg food (Gross et al. 1988) for groups 3 and 4 at the age of 7 day-old till end of the experiment at the age of 42 days.

### Groupings were:

Group 1: Control -ve all (no ammonia exposure and no challenge with NDV).

Group 2: NDV challenge only.

Group 3: Ammonia + ascorbic acid (A + A.A).

Group 4: Ammonia + ascorbic acid + NDV.

6- Sacrification: of 3 birds of each group done at 21, 28, 35 and 42- day- old for organs weight (Gross et al., 1980). Food/gain ratio obtained 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of age. Organs for histopathological examination were collected from the stressed bird before and after A.A. treatment, kept in 10% formalin and processed by the routine paraffin embedding technique, stained with H & E (Bancroft and Cook, (1984). Whole blood and serum were taken weekly 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> week of age for haematological changes (Schlm et al., 1975).

## RESULTS AND DISCUSSION

Results in Table (1) revealed that ammonia stress increased the organs weight during the early exposure then decreased (4<sup>th</sup> week) when compared with the control.

Ascorbic acid showed enhancement effect on organs weight especially immunoorgans (thymus and bursa of fabricius) of the ammonia stressed group compared with challenged group and control one (5<sup>th</sup> week). Double stressed birds with A.A. had more organs weight than the control group except bursa of Fabricius and kidney were lower (5<sup>th</sup> week). Challenged group had more weights than the control.

Late bird response, which might result of inter



## Influence of Ammonia

Table 1: Effect of ammonia and ND challenge on organs weight with and without ascorbic acid and supplement.

Treatment /sge.	Organs weight in gms			Bursa of Fabricum	Kidney	Heart
	Liver	Spleen	Thymus			
At 4 <sup>th</sup> wk. Control -ve	7.3±0.90	0.55±0.02	1.5±0.01	1.6±0.04	1.40±0.05	0.8±0.1
Ammonia (100-pp)-A-	7.05±0.82	7.5±0.30	7.5±0.22	1.45±0.78	1.35±0.87	0.95±0.16
At- 5 <sup>th</sup> -Wk Control	8.4±1.2	0.80±0.02	1.9±0.53	2.6±0.17	2.1±0.8	1.6±0.16
ND challenge	7.9±.6	0.40±0.12	1.96±0.18	8.9±0.08	2.2±0.9	0.7±0.07
Ammonia + ascorbic acid (A+A-A)	11.1±0.45	0.89±0.03	2.4±0.21	2.9±0.90	3.7±1.02	2.5±0.15
Ammonia + ND + <u>Ascorbic acid</u>	11.3±0.2	0.96±0.60	2.2±0.3	1.4±0.05	1.8±0.06	1.6±0.2
At 6 <sup>th</sup> Wk. Control	8.4±1.3	0.82±0.02	1.5±0.08	1.56±0.73	2.87±1.96	0.87±0.02
ND. Challenge	18.8±2.07	1.65±0.07	4.4±1.05	63.±1.43	7.4±1.35	6.3±2.05
Ammonia + ascorbic acid	8.65±1.35	1.06±0.03	2.3±0.75	1.87±0.05	3.30±1.35	1.40±0.07
Ammonia + NDV + ascorbid acid	15.2±3.1	1.76±0.07	3.17±0.75	3.12±0.07	6.3±0.96	2.27±0.035

Means values of 3. birds/each treatment.

struggle to get its homeostasis with their stressors, cleared with the ascorbic acid- ammonia group which got more closet organs weight to the control one. While NDV group still has more organs weight with regard to the thymus, bursa of Fabricius and kidney than rest of the gorups.

From these results, it is clear that, ammonia or NDV alone stress affected bird stress-response (organs weight) in various ways, this response related to the bird adaptation and gaining experiences.

Results in Table (2) showed that, ammonia could increase the mean haematological values than the control one (3<sup>rd</sup> and 4<sup>th</sup> week of age). Ascorbic acid decreased the mean hematological values in ammonia-stressed group (5<sup>th</sup> week). Heterophils/

lymphocytes ratio started high in ammonia group (0.96) then declined linearly with age and with ascorbic acid treatment (0.40). This confirm the bird's ability to readjust its internal environment, confronting the external environmental stressors, and the enhancement effect of ascorbic acid on mitigating those stressors, with subsequent improvement of bird performance (MacFarlane and Curties, 1989 and Gross, 1992).

Results of Table (3-A) indicated that ammonia stressed birds had less food utilization, body weight gain and food/gain ratio than the control (2<sup>nd</sup> and 3<sup>rd</sup> week of age). This continuous ammonia stress affected the bird's ability to gain access feeders and consequent less food:gain ratio till the 4<sup>th</sup> week of age than the rest of groups.



Table 2: Mean hematological values of stressed birds with ammonia and NDV, with and without Ascorbic acid

Age Wk. Group	Hb/dl	PCV %	Rbcx10 <sup>6</sup> /mm <sup>3</sup>	WBCx10 <sup>3</sup> /mm <sup>3</sup>	Hetrophil No.	Lymphoc ytes No.	H/L ratio	Serum protein gvd/dl.
3rd C	8.4±1.05	28.0±4.0	1.8±0.33	55.5±10.5	49.0±9.0	95±0.0	0.40±0.02	3.0±1.90
A	9.75±0.25	32±0.25	2.3±0.11	57.5±11.5	9.0±3.0	95±3.0	0.96±0.31	3.40±0.70
4th C	8.5±0.9	30±2.1	1.6±0.04	27.5±2.5	40.0±6.00	98.0±3.00	40.0±0.08	4.50±0.91
A	7.5±1.5	31.0±3.0	1.8±0.03	75.0±7.0	75.0±1.5	95.5±1.5	0.79±0.01	6.0±0.3
5th C	7.3±0.2	30.0±1.31	1.5±0.05	30.0±1.86	48.0±2.1	90.0±0.23	0.53±0.07	4.4±0.91
NDV	9.0±1.42	31.5±0.03	2.18±0.05	37.0±3.6	50.0±1.7	98.0±1.5	0.51±0.06	6.75±1.15
A+AA	9.0±0.98	29.5±1.87	2.09±0.05	35.0±3.1	35.0±0.81	92.0±2.3	0.30±0.013	4.4±0.80
A+ND+AA	9.0±0.20	32.0±0.15	2.31±0.13	45.0±2.6	60.0±1.3	94.0±1.76	0.64±0.073	6.34±0.42
6th C	9.0±0.03	32.5±1.2	1.52±0.03	60.0±2.1	65.0±1.00	98.0±2.1	0.66±0.03	5.2±0.2
NDV	9.5±0.56	35.0±0.90	2.7±0.01	66.0±3.5	57.0±0.4	98.0±1.6	0.58±0.17	5.4±0.7
A+AA	9.0±0.31	32.5±0.8	2.5±0.04	62.0±2.9	42.0±2.1	95.0±3.7	0.44±0.13	6.5±0.2
A+ND+AA	9.5±0.81	35.5±0.70	2.8±0.09	60.0±4.3	60.0±1.80	99.0±2.8	0.61±2.1	4.8±0.4

N.B= C= Control

A= Ammonia / A.A.= Ascorbic acid/

ND Newcastle-disease virus challenge.

Challenged birds got less food/gain ratio than the ammonia treated A.A. and double stressed-treated with A.A. This results confirm the growth enhancement effect of A.A. either with single or double stress (viricidal effect with NDV), this effect is related to the quality of husbandary, length of supplementation and age of bird (Niekerk et al., 1989). Also this improvement ensure the bird's ability to response well with managemental breaks and to modulate its endogenous ascorbic acid resources at certain level of stress (Pardue and Thaxton, 1986).

Data in Table (3-B) showed that, mortality rate started high in challenged group alone than with double stress with ascorbic acid (4-day post-challenge), then ran together at the same rate than the A.A. treated groups. Ascorbic acid showed an immune enhancement effect which confirme by Dahl and Degre, (1986) and White et

al. (1986).

Histopathological examination revealed that the ammonia stressed birds showed focal necrobiotic changes in the liver and kidney. The heart suffered from serofibrinous pericarditis. The lesions in the lymphoid organs characterized by lymphocytic depletion, the thymus showed focal disintegration of cortical thymocytes forming starry sky appearance (Fig. 1a) and consequently there was lymphocytic depletion in the spleen (Fig. 2-a) and bursa of Fabricius. Christopher (1975) confirms these changes with high NH<sub>3</sub> concentration.

Ascorbic acid treated group showed an improvement in the degree of lesions in parenchymatous organs (Table 4), lesser lymphocytic disintegration and depletion of lymphoid organs, thymus (Fig. 1-b), bursa of



Table 3: Mean values of food intake, body weight gain and gain/food ratio of double stressed birds with ammonia and Newcastle disease virus either treated or not with A.S.

Age in Weeks.	Groups	Food intake (gm/bird)	B.Wt/Gain (gm/bird)		F/Gain ratio
1st	C	112	126	93	1.20
	C	214	285	149	1.44
2nd	A	190	260	134	1.42
	C	444	570	286	1.55
3rd	A	410	500	240	1.71
	C	540	870	300	1.8
4th	A	555	785	285	1.94
	C	600	1200	330	1.82
5th	ND	580	1050	265	2.20
	A+AA	610	1080	295	2.06
	A+ND	630	1055	270	.30
	AA				
6th	C	790	1500	300	2.63
	ND	780	1300	250	2.1
	A+AA	800	1370	290	2.75
	A+ND	788	1320	265	2.97
	AA				

C= Control  
 A= Ammonia                      A.A. Ascorbic acid.  
 ND Newcastle-disease virus challenge.

Table 3 B: Mortality rates post-NDV challenge with or without A.A. supplementation

Days post-challenge	Groups	Mortality %
4	ND	10
	A+ND+A.A	5
5	ND	5.5
	A+ND+A.A	5.5
6	ND	23.5
	A+ND+A.A	21.3
7	ND	7.6
	A+ND+A.A	7.1

Table 4: Score of histopathological lesions of internal organs of stressed birds and after treated with ascorbic acid.

Groups	Necrobiotic changes	Liver		Kidney	Heart		Lymphocytic depletion		
		Periportal lymphocytic aggregation	Hyperplasia of kuffer cells	Nephrosis	Focal myocarditis	Pericarditis	Thymus	Spleen	Bursa
C	-	+	-	-	-	-	-	-	-
A	++	+	-	++	+	++	++	+++	++
A+A.A	+	+	++	+	-	-	-	++	+
ND	+++	+++	+	+++	++	-	+++	+	+++
A+A.A + ND	++	++	+	+	-	-	+	-	-

C= Control  
 A= Ammonia                      A.A. Ascorbic acid, ND Newcastle-disease virus challenge.





Fig. (1-a): Thymus of a chicken treated with ammonia showing desintegration of cortical thymocytes giving starry sky appearance. H & E x 250.

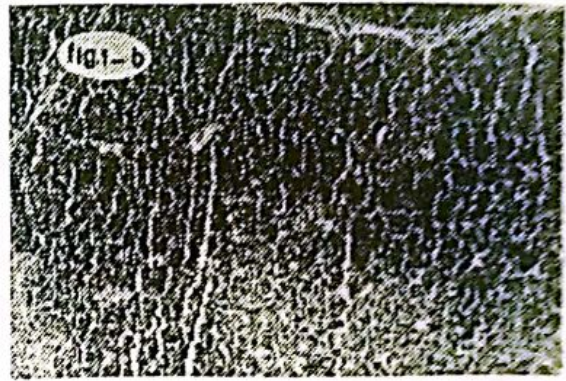


Fig. (1-b): Thymus of a chicken group 3 showing improvement of the cortical thymocytes desintegration. H & E x 250.

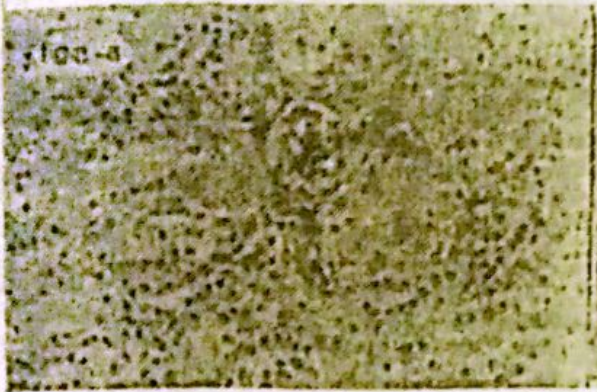


Fig. (2-a): Spleen of a chicken treated with ammonia showing lymphocytic depletion and vascular congestion. H & E x 250.

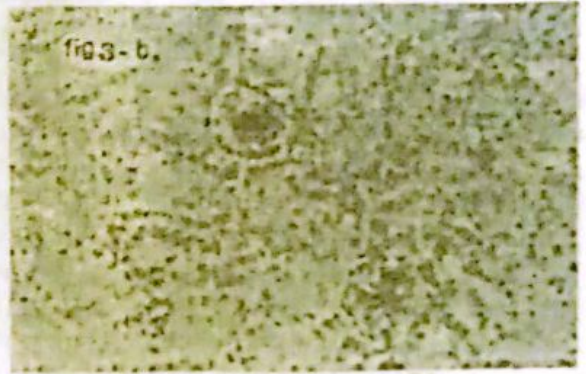


Fig. (2-b): Spleen of a chicken treated in group 3 showing improvement of lymphocytic depletion with reticular cells hyperplasia. H & E x 250.

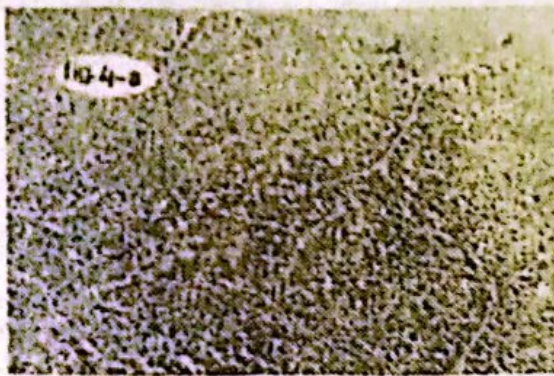




**Fig. (3-a):** Kidney of a chicken in group 2 showing severe nephrosis where the tubular epithelium suffer from necrobiotic changes. H & E x 400.



**Fig. (3-b):** Kidney of a chicken in group 4 showing improvement in the degree of necrobiotic changes of the tubular epithelium. H & E x 400.



**Fig. (4-a):** Bursa of Fabricius of a chicken in gorup 2 showing lymphocytic depletion and atrophy of bursal follicles. H & E x 1050.



**Fig. (4-b):** Bursa of Fabricius of a chicken in gorup 4 showing improvement of lymphocytic depletion. H & E x 100.



Fabricius and spleen with reticular cells hyperplasia (Fig. 2-b). The reticular cells hyperplasia in the spleen confirm the enhancement of ascorbic acid to reticuloendothelial cells activity as recorded by Gross et al., (1988).

The challenged group only showed focal hepatic necrosis associated with inflammatory cell reaction mainly lymphocytes and heterophils. In addition to increase lymphocytic aggregation around portal areas. In Kidney, there was severe extended nephrosis Fig. (3-1) and in the heart focal areas of myocarditis, characterized by mononuclear and heterophilic cells aggregation. The lymphoid organs showed marked lymphocytic depletion especially the bursa of Fabricius Fig. (4-a). The thymus had severe cortical lymphocytic depletion with congestion and haemorrhage in the medulla.

Challenged treated group with A.A. had improvement in the degree of lymphoid organs and parenchymatous organs lesions Fig. (4-b) and Fig. (3-b).

However, liver or kidney damage due to long-term stress might give clues on how specific stressors alter structures or functions to shunt resources away from production processes (Essa et al., 1985).

Progress in poultry environment would be enhanced if more were known about how several stressors acting together affecting poultry overall well-being and performance.

Poultry hygienist should be alert to any deviation of management, birds behavioural responses start with determining the nature, intensity and timing of stress (early or late life). Measuring of bird stress-response (H/L ratio) and trial to mitigate the stress effect as possible using proper dose, duration and application of ascorbic acid.

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