BIOCHEMICAL AND IMMUNOLOGICAL STUDIES ON THE EFFECT OF *NIGELLA SATIVA* ON AFLATOXIN IMMUNOSUPPRESSED CHICKENS AGAINST NEW CASTLE DISEASE VIRUS VACCINE

By

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ABSTRACT

The experiment was designed to study the immunological and biochemical effect of *Nigella sativa* and aflatoxin on chicken vaccinated with inactivated Newcastle disease virus vaccine. The immunological effect was measured by Haemagglutination Inhibition test (HI test) and serum biochemical changes were indicated by measuring total protein and fractionation of protein by electrophoresis. The results showed clearly that aflatoxin was functionally alter the immune system of the bird by immunosupression giving HI GM titer 3.2 comparing with control group that gave 6.4 at 21 days post-vaccination and giving gammaglobulins protein fraction 0. 65g/dl comparing with control group that gave 0.91g/dl at 21 days post- vaccination. On other hand *Nigella sativa* was a potent immunostimulant where it gave HIGM titer 8. 2 and gammaglobulin fraction 1.11g/dl at 21 days post NDV vaccination and it could counter the immunosuppressive effect of aflatoxin.

INTRODUCTION

Immunosuppressive state in poultry farms constitute a major problem facing the rapidly expanding poultry industry allover the world. There are several factors that have been incriminated causes of this condition. Immunological alteration of the immune system of the birds by mycotoxins play an important role as a cause of this condition, one of the most important toxin is aflatoxin. Aflatoxin is a toxic product of fungal growth produced primarily by the moulds, *Aspergilus flavus* and *parasiticus* in cereal grains, particularly corn in which its spores germinate during storage. Studies have shown that aflatoxins are immunosuppressive and its investigation in feed has resulted in decrease immunity in vaccinated birds (**Hamilton, 1982; Biresova *et al.*, 1997 and Gabal and Azzam, 1998**). Potentiation of immune response

in poultry can be occurring by some additives, however the routine use of drugs to modify or modulate animal immune competence as a part of therapeutic management of specific clinical condition is still at a very preliminary stage in veterinary medicine (**Bronder *et al.*, 1991**). Moreover there is a herbaceous plant known as *Nigella sativa* which is a member of Ronunculacor family that have the ability to increase the immunity and maintain a good health condition (**Abd-El Aoi and Attia, 1993**).

MATERIAL AND METHODS

I- Materials:

1- Virus: A velogenic viscerotropic NDV obtained from Veterinary Serum Research Institute ND Unite, Abbasia, Cairo was used as a challenge virus.

2- Virus vaccine: Combined ND and IBD inactivated virus vaccine produced by Rhone Merieux 17 RUE Bourgelat 69002 LYON France Lot. No. 70 g 531 was used

3- Fertile chicken eggs: 9 – 11 day- old Commercial Embryonated Chicken Eggs (ECE) were purchased from commercial source were used for preparation of HA NDV antigen and virus reisolation from challenged birds.

4- Experimental Chickens: A total of one hundred day- old Fayoumi-chickens obtained from EL- Azab poultry farm were used.

5- *Nigella sativa* seeds: They were purchased and freshly crushed and added to the balanced commercial ration in a dose of 1%.

6- Aflatoxin B1: It purchased from Sigma Company and added by a dose of 0.25 mg/Kg diet according to (**Giambrane *et al.*, 1978**). Aflatoxin B1 was dissolved in chloroform and spread on the ration, then evaporated over night at 50° C.

7- Ration: Balanced commercial ration obtained from commercial source without any food additives was used.

II- Methods:

1- Haemagglutination Inhibition Test (HI Test): It was carried out on collected sera according to the standard procedures described by **Mojujobe and Hitchner, (1977)** and the Geometric mean titer was calculated as described by **Anon (1971)**.

2- Vaccination of chickens: It was applied at 21 days of age (where maternal antibodies are negligible) by a dose of 0.3 ml/ bird intramuscularly as described by the vaccine manufacture.

3- Serum biochemical studies: Total proteins were measured according to **Doumas (1975)** and fractionation of proteins was adopted by electrophoresis using the technique of **Ogowa, (1968)** on collected serum samples post-vaccination.

4- Challenged test: Chickens in all groups were challenged orally at 21 days post- vaccination with a virulent NDV of a titer 10^6 EID_{50} / ml by a dose of 0.1 ml according to **Abou- Elkhair *et al.*, (1998)**.

5- Statistical Analysis: Analysis of variance was applied according to **Snedecor and Cochran, (1976)**.

The experimental design:

Total one hundred one- day- old Fayomi chickens were divided into four groups. All birds were housed in separate pens and fed on a commercial balanced ration adlibitum consumption.

Group I: Fed on ration mixed with *Nigella sativa* 1%.

Group II: Fed on ration containing Nigella sativa 1% and Aflatoxin B1 0.25 mg/Kg diet.

Group III: Fed on ration mixed with aflatoxin B1 at dose of 0. 25mg/kg diet daily.

Group IV: Fed on normal ration as a control group.

Chickens in all groups were kept under observation for 45 days and serum samples were collected weekly and subjected to HI test to detect maternal NDV antibodies until declined and biochemical parameters. Moreover, chickens in all groups were vaccinated with combined ND and IBD inactivated viral vaccine at 21 days of age (when NDV maternal antibody curve is decline) by a dose of 0.3 ml/ bird intramuscularly.

Chickens in all groups were challenged orally at 21days post- vaccination with virulent NDV according to (**Abou- Elkhair *et al*.,1998**) and kept under observation for 15 days. Dead birds and those showing symptoms examined for P.M. lesions within this period.

RESULTS

Table (1): HI GM titer for NDV maternal antibody in different groups of chickens

	Treatment		HI GM titer			
	N.S	AF.BI	Zero day	7 day	14 day	21 day
I	+	-	7.2	5.0	2.6	0.2
II	+	+	7.2	4.8	2.6	0.2
III	-	+	7.2	4.8	2.6	0.2
IV	-	-	7.2	5.0	2.4	zero

+ = Treated - = non Treated

From table (1) it is clear that HI maternal antibody for NDV was declining and became negligible at 21 day of age without significant difference between means.

Table (2): HI GM titer for NDV vaccination in different groups of chickens

Groups	HI GM titer		
	7 day post-vaccination	14 day post-vaccination	21 day post-vaccination
I	0.8	6.0	8.2
II	0.2	5.0	6.2
III	0.2	2.8	3.2
IV	0.4	5.4	6.4

Form table (2) it is clear that *Nigella sativa* (group I) gave higher HI GM titer (8.2) at 21 days post vaccination with inactivated NDV vaccine while aflatoxin B1 (groupIII) gave low immune response (HIGM titer 3.2) at 21 days post-vaccination. The group II fed on ration containing *Nigella sativa* 1% and Aflatoxin B1 0. 25mg/Kg diet gave HIGM titer 6.2 while the control group gave HIGM titer (6.4), this result indicates that the *Nigella sativa* could counter the immunosuppressive effect of aflatoxin.

Table (3): Serum protein fractions in different groups of chickens post-vaccination with Newcastle disease vaccine.

Groups	1st Week							2nd Week							3rd Week						
	TP G/dl	Alb G/dl	Alpha G/dl	Beta G/dl	Gamm G/dl	Total glob. G/dl	A/G	TP G/dl	Alb G/dl	Alpha G/dl	Beta G/dl	Gamma G/dl	Total glob-ulin. G/dl	A/G	TP G/dl	Alb G/dl	Alpha G/dl	Beta G/dl	Gamma G/dl	Total glob-G/dl	A/G
N.S	3.33 ± 0.27	1.54 ± 0.08	0.49 ± 0.02	0.49 ± 0.03	0.80 ± 0.02	1.78	0.9/1	A 3.91 ± 0.13	A 1.85 ± 0.02	A 0.60 ± 0.02	A 0.35 ± 0.02	A 1.10 ± 0.07	A 2.05	0.9/1	A 3.89 ± 0.01	A 1.60 ± 0.04	A 0.50 ± 0.01	A 0.59 ± 0.02	A 0.11 ± 0.01	A 2.24	0.7/1
N.S + AF	3.34 ± 0.11	1.54 ± 0.01	0.41 ± 0.01	0.45 ± 0.05	0.94 ± 0.06	1.88	0.9/1	B 3.07 ±0.04	B 1.50 ± 0.05	A 0.53 ± 0.04	A 0.32* ± 0.02	B 0.87 ± 0.01	B 1.62	0.9/1	B 3.02 ± 0.07	B 1.28 ± 0.05	A 0.44 ± 0.01	B 0.43 ± 0.01	B 0.87 ± 0.04	B 1.74	0.7/1
AF only	3.34 ± 0.18	1.56 ± 0.02	0.49 ± 0.06	0.45 ± 0.05	0.84 ± 0.06	1.78	0.8/1	B 2.97 ± 0.04	B 1.27 ± 0.03	B 0.42 ± 0.02	B 0.51 ± 0.01	B 0.78 ± 0.02	B 1.7 1	0.8/1	BC 2.59 ± 0.03	B 1.16 ± 0.02	B 0.35 ± 0.01	B 0.40 ± 0.01	C 0.65 ± 0.01	C 1.43	0.8/1
C	3.26 ± 0.12	1.55 ± 0.02	0.49 ± 0.49	0.41 ± 0.03	0.81 ± 0.04	1.82	0.8/1	B 3.35 ± 0.17	A 1.61 ± 0.06	B 0.39 ± 0.05	A 0.44 ± 0.01	A 0.90 ± 0.05	B1.73	0.9/1	B 3.20 ± 0.01	A 1.59± 0.04	A 0.49 ± 0.01	B 0.31 ± 0.01	A B 0.91 ± 0.02	B 1.71	I/1
LSD 5%	0.489	0.279	0.116	0.156	0.212	0.297		0.489	0.279	0.116	0.156	0.212	0.297		0.489	0.279	0.116	0.156	0.212	0.297	
1%	0.725	0.406	0.169	0.226	0.309	0.432		0.725	0.406	0.169	0.226	0.309	0.432		0.725	0.406	0.169	0.226	0.309	0.432	

* Data have the same litter in the same column are non- significantly different. Data have different litter in the same column are significantly different at p<0.5.
NS, (group I), fed on Nigella sativa; NS + AF. (group II), fed on Nigella sativa and aflatoxin B1. AF, (group III). fed on Aflatoxin B1 only and C (group IV) non treated.
TP = Total protein Alb = Albumin Alpha. G =Alphaglobulin Gamma G = Gammaglobulin A/G = Albumin/ globulin ratio

From table (3) it is clear that there was no significant difference in all groups in protein fractions in the first week. On second and third week there was significant decrease in total protein and albumin in all groups especially group III (which fed on ration containing aflatoxinB1) as compared with group I (which fed ration containing Nigella sativa 1%). Also there was a significant decrease in alpha, beta globulins and gamma globulins in group III as compared with group I and group IV (control group).

Table (4): Challenge of vaccinated chickens with virulent NDV

Group	Clinical symptoms	P.M. examination	Virus resolution
I	Apparently normal	Apparently normal	- ve
II	Apparently normal	Apparently normal	- ve
III	Birds showed marked depression, conjunctivitis, and respiratory signs in the form of gasping and nasal discharges. Four birds were died out of 20. One at 3rd day and three at 5 days post-challenge	Severe catarrhal inflammation of mucous membrane of respiratory and digestive tract mainly in gizzard and proventriculus which showed peticeal hemorrhage	+ ve
IV	Apparently normal	Apparently normal	- ve

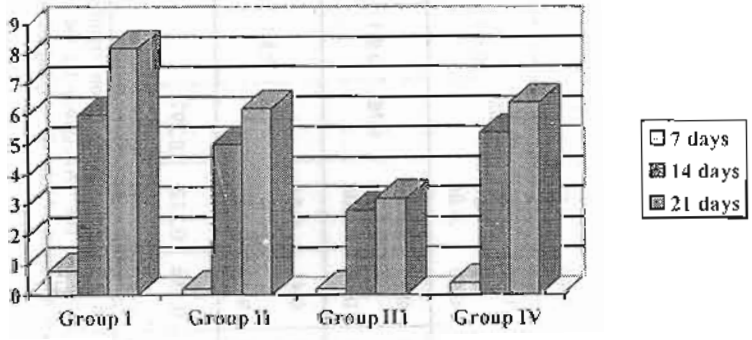

Fig. (1): HI GM titer for NDV vaccination in different groups of chickens

الملخص العربي

دراسة بعض التغيرات الكيميائية الحيوية والمناعية لتأثير حبة البركة على الدواجن المحصنة بلقاح النيوكاسيل والمسممة بالأفلاتوكسين

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صممت هذه التجربة لدراسة التأثير الكيميائى الحيوى والمناعى لحبة البركة مع الأفلاتوكسين الدواجن المحصنة بلقاح النيوكاسل. تم قياس التغيرات المناعية بواسطة HI test. وقياس التغيرات الكيميائية بواسطة قياس البروتين الكلى وعمل الفصل الكهربائى لها. وقد أظهرت النتائج أن هناك تأثير تثبيط مناعى للأفلاتوكسين على الجهاز المناعى للطيور . وعلى الجانب الأخر حبة البركة كانت مقوية ومنشطة للجهاز المناعى وتضاد التأثير التثبيطى للأفلاتوكسين للدواجن بعد تحصينها بلقاح النيوكاسل.