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## EFFECT OF AFLATOXINS ON NEWCASTLE DISEASE VACCINATION IN BROILER CHICKENS

By

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دراسة تأثير سموم الافلاتوكسينات على التحصين ضد  
مرض النيوكاسل في بداري الدجاج

كمال الزناتى ، سعد المرافسى ، طلبه يونس ، بهيت سالم

أمكن في هذا البحث عزل إحدى وعشرين معزولة لفظره اسبرجيلس فلافس من عشرة عينات من علائق الدواجن والتي جمعت من مزارع الدواجن المختلفة بمحافظة أسيوط وذلك باستخدام طريقة التخفيف والوسط الغذائي شابكس آجار والتحصين عند ٢٨م . هذا وقد أختبرت مقدرة المعزولات الفطرية المعزولة على إنتاج سموم الافلاتوكسينات باستخدام التخمير في المزارع السائلة ، وقد أظهر التحليل الكروماتوجرافي وجود سموم الافلاتوكسينات  $B_1, B_2, G_1$  &  $G_2$  في مستخلص ثلاثة معزولات منها ، وكان أكبر تركيز لخليط سموم  $B_1, B_2, G_1$  &  $G_2$  المنتجة يعادل ٢٨٠ مجم لكل لتر وسط غذائي . وقد تمت الدراسة تأثير تركيزات مختلفة ( صفر ، ٨ ، ١٦ ، ٢٢ ، ٤٨ ميكروجرام طائر ) من سموم الافلاتوكسينات على التحصين ضد مرض النيوكاسل في بداري الدجاج عند عمر أربعة أيام ، ١٨ يوماً باستخدام عترة الهنتشر بهعتره اللاسوتا على التوالي وكذلك عند عمر ٢٥ يوماً باستخدام عترة الكوماروف العضلي . هذا وقد وجد إنخفاض في مستوى قياس الأجسام المناعية لتحصينات النيوكاسل باستخدام إختبار الازن الدم المضاد عند اليوم العاشر ، السابع عشر والرابع والعشرين بعد المعاملة وعدم تأثير الوقاية وذلك بإختبار تحدى المناعة عند نهاية التجربة .

### SUMMARY

Twenty-one isolates of *Aspergillus flavus* Link were isolated from ten poultry feed stuff samples collected from different poultry farms at Assiut governorate using the dilution plate method on glucose-Czapek's agar medium incubated at 28°C. All *A. flavus* isolates were screened for their ability to produce aflatoxin on liquid medium culture. Chromatographic analysis revealed that aflatoxins  $B_1, B_2, G_1$  &  $G_2$  were present in the chloroform extracts of three isolates. The higher aflatoxin concentration produced by the different isolates was 280 mg per liter.

A different concentrations of aflatoxin (0, 8, 16, 32 and 48 ug/bird) were given daily for two weeks to five groups of 15 birds of five weeks old. These chickens were vaccinated against Newcastle disease virus (NDV) at 4<sup>th</sup> and 18<sup>th</sup> day age with Hitchner  $B_1$  and

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LaSota strain respectively and at 35th day with komarov strain. Adverse effect on humoral immunity to ND vaccination was observed as measured by hemagglutination inhibition test (HI) at 10th, 17th and 24th days post treatment, and no effect on protection as indicated by challenge test at the end of experiment.

## INTRODUCTION

Aflatoxin ( $B_1$ ,  $B_2$ ,  $G_1$  &  $G_2$ ) are common mycotoxins found in feed stuffs (DOUPNIK, 1977; EL-MARAGHY and SALEM, 1986) that cause a variety of adverse effects in poultry (VOHARA, 1978). The most important and biologically active component of these toxins is aflatoxin  $B_1$  (BUSBY and WOGAN, 1981). The inhibitory effect of aflatoxin on antibody production has been demonstrated in chicks (THAXTON, *et al.* 1974) in contrast, other research has shown no effect of aflatoxin on antibody formation in chickens and turkeys (ADINARAYANAIAH, *et al.* 1973; RICHARD, *et al.* 1973 & PIER, *et al.* 1970).

The object of the present study was to clarify the effect of aflatoxin on vaccination against Newcastle disease virus.

## MATERIAL and METHODS

Ten feed stuff samples (one kg/each) were collected from different poultry farms at Assiut Governorate (egg layer ration, six samples; broiler fattening feed, three samples; concentrates for broiler, one sample).

### Isolation of *Aspergillus flavus* species

This was made by using the dilution plate method as described by CHRISTENSEN (1963) but with some modification as described by MOUBASHER, *et al.* (1979) using glucose-Czapek's agar medium + rose bengal (1/15000) as a bacteriostatic agent (SMITH and DAWSON, 1944) and incubation at 28°C for 7 days.

### Medium for screening

Different *Aspergillus flavus* isolates were screened for aflatoxin production using a liquid medium, which contained: Sucrose, 30; Peptone, 10;  $NaNO_3$ , 2.0;  $K_2HPO_4$ , 1.0; Yeast extract, 1.0;  $KCl$ , 0.5;  $MgSO_4$ , 0.5;  $FeSO_4$ , 0.01 (mg/L of distilled water) the initial pH of the medium was 5.5. The medium was sterilized by autoclaving for 20 min. at 121°C.

### Cultivations

Inocula of 1- cm diam. agar disks from one week-old cultures obtained after subculturing the isolates on Czapek's agar medium, were transferred to 500 ml Erlenmeyer flasks, each containing 100 ml of the medium. Flasks were incubated as stationary cultures at 28°C for 10 days.

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Extraction and analysis of aflatoxin of different isolates

At the end of incubation period, the content of each flask was homogenized with 100 ml of chloroform for 5 min in a high speed blender (16000 rpm). Extraction was repeated three times with 100 ml portions of chloroform. The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulphate, filtered and concentrated to near dryness on a rotatory evaporator. The residue was dissolved in 2 ml of chloroform-methanol (1:1 vol/vol). Thin layer plates of silica gel (Kiesel gel G, Merck) plates (0.5 mm in thickness), activated for one hour at 110°C was spotted with 50  $\mu$ l of the extract. The plates were developed with chloroform-methanol (98:2 vol/vol). Aflatoxins were identified by comparison with appropriate reference standards and their blue and green fluorescences under long UV light.

Confirmatory test for aflatoxins

Chemical confirmatory test for aflatoxin, B<sub>1</sub> by direct formation of hemiacetal B<sub>1</sub>a on thin-layer plate before chromatography as described by PRZYBYLSKI (1975) was used. The unknown and standard aflatoxin B<sub>1</sub> spots are treated with trifluoroacetic acid directly on the same plate. After reaction the plate is developed as usual and examined under UV light. The hemiacetal derivative has a blue fluorescence at a lower RF than B<sub>1</sub>.

Aflatoxin content of extract was estimated using the technique described by COOMES, et al. (1965).

Chicks

One-day old Fayoumi chicks were obtained from commercial flock reared under strict conditions. Feed and water were available and libitum and shown to contain no aflatoxin.

Vaccination programme

Routine programme of vaccination in commercial poultry farms were applied at 4th, 18th and 35 days age using Hitchner B<sub>1</sub> and LaSota vaccine respectively in the drinking water in the first two vaccinations followed by komarov intramuscularly (i/m) vaccine at 35 days of age in all five groups.

Administration of aflatoxin

The chicks were divided into 5 groups of 15 birds each (2, 3, 4, 5 groups) to receive 8, 16, 32, 48  $\mu$ g aflatoxins mixture (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>) bird respectively daily for 2 weeks by injection through crop at 35 days age. The first group was left without addition of aflatoxin (0) to serve as control.

Hemagglutination inhibition (HI) and challenge test:

Antibodies to NDV were measured by HI (BRUGH, et al. 1978) at 5, 10, 17 and 24 days post inoculation by aflatoxin. For challenge test, bird received 0.05 ml of 1:1000

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dilution of local velogenic NDV strain (ELD<sub>50</sub> 10<sup>9.3</sup>/ml) which injected intramuscularly, morbidity and mortality were observed for 2 weeks.

## RESULTS

Twenty-one cultures of *Aspergillus flavus* Link were isolated in the present investigation from poultry feed stuff samples (egg layer ration, nine isolates; broiler fattening feed, seven; and concentrates for broiler, five).

All the 21 pure cultures of *A.flavus* were screened for their ability to produce aflatoxin. Thin-layer chromatographic analysis revealed that aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub> were present in the extract of three isolates (egg layer ration, 2 isolates; and concentrates for broiler, one isolate). The total concentration of aflatoxins in the later isolate was high and equal to 280 mg/L. This isolate was used as aflatoxin producer in the present investigation. An additional confirmatory test for aflatoxin B<sub>1</sub> was made. Aflatoxin B<sub>1</sub> was successfully derivated to give the hemiacetal B<sub>1</sub>a (PRZYBLSKI, 1975).

All groups given aflatoxins for the 1<sup>st</sup> five days had no adverse effect on hemagglutination inhibition test (HI) titer as compared by control group, 10 days after aflatoxins administration HI test revealed no effect in the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> groups while the 5<sup>th</sup> group showed drop in HI liter by 2.1 log 2. The 4<sup>th</sup> and 5<sup>th</sup> groups at 17<sup>th</sup> and 24<sup>th</sup> day post aflatoxin administration showed decrease in HI titers ranged from 2.8 - 3.3 log 2 as compared with the control group.

In all treated groups at 5<sup>th</sup>, 10<sup>th</sup>, 17<sup>th</sup> and 24 days post aflatoxin administration, no clinical signs or deaths were observed after challenge with a velogenic ND strain.

## DISCUSSION

The highly toxic and carcinogenic metabolites (aflatoxins), is a generic term that refers to one or more of four principal metabolites which produced by certain strains of *Aspergillus flavus* and *A.parasiticus*. These are aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>. *A.flavus* is universally distributed in the environment and aflatoxin contamination in foods and feeds has been detected in all parts of the world (FAO, 1979, VENDANAYAM, et al. 1971 & EL-KADY, et al. 1984). *A.flavus* contamination of feed stuffs were previously studied in laboratory of physiology of fungi Faculty of Science, Assiut University (EL-KADY, et al. 1984, EL-MARAGHY and SALEM, 1986 & YOUSSEF, 1986). They reported that *A.flavus* was the most common *Aspergillus* species on the feed stuffs tested. A wide range in the ability of *A.flavus* strains to produce aflatoxin was reported soon after SARGEANT, et al. (1961) first associated the toxin with *A.flavus* Link. EL-KADY, et al. (1984) and YOUSSEF (1986) found that 33% and 37% of *A.flavus* strains isolated from cotton seeds and soybean seeds produced aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub> respectively.

Data reported herein indicated that aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>) given at 5-week-old Fayoumi chickens daily for 2 weeks at a concentration of 0, 8, 16, 32 and 48 ug/bird

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caused only immunodepressant as indicated by HI test in the last two groups while no effect was noticed in the other two treated groups (8, 16, ug/bird). Our results are in agreement with THAXTON, et al. 1974 and ILGAS, 1985 and in contrast other researchs has shown no effect of aflatoxin on antibody formation (ADINARAYANAIAH, et al. 1973, RICHARD, et al. 1973, PIER, et al. 1970 and GIAMBRONE, et al. 1985).

In this study the challenged birds showed no clinical signs or deaths in all treated groups. This was in agreement with GIAMBRONE, et al. 1985 who gave aflatoxin B<sub>1</sub>.

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## SUMMARY