3rd Scientific Congr. of Egypt. Soc. For Anim. Manag. 28-29 Oct.,2008 :20- 55

EFFECT OF DIETARY AFLATOXIN B1, EIMERIA TENELLA AND MYCOPLASMA GALLISEPTICUM ON THE UMORAL IMMUNE RESPONSE TO AVIAN INFLUENZA INACTIVATED VACCINE IN BROILER CHICKENS

By

K. MADIAN

Dept. of Poultry Dis., Faculty of Vet. Med., Cairo University

SUMMARY

This study aimed to study the effects of aflatoxin B1 in feed (dietary aflataxin),*Eimeria tenella*, and *Mycoplasma gallisepticum* infections on the performance and humoral immune response to avian influenza inactivated vaccine in broiler chickens. Two hundred and forty one– day old broiler chicks were used and randomly assigned to six equal experimental groups, group A was kept as control negative (non– vaccinated/non–treated),group B was left as control positive (vaccinated/non–treated),group C fed on diet contaminated with aflataxin at dose level of 2 mg/kg feed from one day old till 42 days of age, group D was inoculated orally at the 15th day of age with $2x10^3$*E.tenella* sporulated oocysts / bird, group E was inoculated orally with the same dose of *E.tenella* sporulated oocysts as group D but at the day 21 of age and group F was intranasally infected with *Mycoplasma gallisepticum* at dose level of 8×10^4 colony forming unit (cfu) / bird, on day 15th of age, all birds in all groups except those in group A were vaccinated with AI inactivated vaccine via intramuscular injection. Humoral immune response to AI vaccine was evaluated by determining antibody titres by using both haemagglutinating inhibition (HI) test and ELISA test. Weekly for four weeks post AI vaccination. Growth performance in the experimental groups was assessed by recording body weight, body weight gain and feed conversion ratio. At the end of the experiment. The results showed that haemagglutination inhibition (HI) and ELISA antibody titres to AI vaccine were significantly (P<0.0s) reduced in the group C (aflatoxin–treated birds, group D,E (*Eimeria tenella* – infected birds) and in group F (*Mycoplasma gallisepticum* infected birds) when compared to those in group B (vaccinated / non–treated). Statistical analysis of the results revealed that the effect of aflatoxin on the humoral immune response to AI vaccine was highly significant than the effect of *E.tenella* and *Mycoplasma gallisepticum* respectively. Also, the effect of early infection with , *Eimeria temella* (at the 15th days of age) on antibody titres was substantial than the infection with E.tenella at 21 day – old. This study indicated that

dietary aflatoxin, *Eimeria tenella,* and *Mycoplasma. gallisepticum* reduce the humoral immune response to AI vaccine in broiler chickens, i.e have an immunosupreesive effect**.**

INTRODUCTION

Avian influenza (AI) is one of the greatest public health concerns to have emerged from the animal reservoir in recent time (**Capua, 2007**). Over the past five years there has been a sharp increase in the number of outbreaks of A. I. in poultry. It has been calculated that the impact of AI on the poultry industry has increased 100-fold, with 23 million birds affected in the forty year period between 1959 and 1998 and over 200 million from 1999 to 2006 (**Capue, and Alexander 2004**).

[

In fact, from the late 1990s, AI infections have assume a completely different profile both in the veterinary and medical scientific communities. In recent times some outbreaks have continued to be of only minor relevance while others, such as the ongoing Eurasian – African HSN epidemic and outbreaks that occurred in **Italy (1999-2000), Netherland (2003**), **Canada (2004)** and **Egypt (2006)** have led to devastating consequences for the poultry industry, negative repercussions on public opinion and in some cases have created significant human health issues, including the risk of generating a new pandemic virus for humans via the avian – human link (**Capua, 2007**). Highly pathogenic avian influenza viruses (HPAIVs) typically produce a similar sever, systemic disease with high mortality in chickens and gollinacious birds **(Swayne, 2007**). Twenty six epizootics of HPAI have occurred in the world since 1995, the Largest of these outbreaks has been the H5N1 subtype which caused problems in poultry and some wild birds in over 60 countries of Asia, Europe and Africa since beginning 1996 **(Swayne, 2007**). In the face of disease outbreaks in poultry and the potential threat to humans caused by the highly pathogenic avian influenza viruses (HPAIVs) of H5N1 subtype, vaccination programs by using inactivated, vaccine in conjunction with biosecurity measures of high standard are two main options in controlling and ultimately in eradicating the disease (**Capua et al.2004, Capua and Maarangon, 2006., Van Der Goot et al., 2005**). Also vaccination of poultry is being recommended by FAO and OIE as supplementary measure. That can contribute to the control of highly pathogenic H5N1 AI in poultry in affected countries (**INFOSAN, 2005**).

Vaccination is a potentially powerful tool to support control and eradication program by increasing the resistance of birds to field strain (challenge) and reducing the levels (amount) and duration of viral shedding from vaccinated birds in the environment (**Capua ,2007**) prophylactic vaccination for viruses of the H5 and H7 subtypes perceived as a tool to maximize biosecurity measures when risk of exposure is high and reduce the number of secondary outbreaks, thus minimizing the negative effects on animal welfare and potential economic losses in areas where ht density of the poultry population would otherwise result in uncontrollable spread without preemptive culling (**Capua and Marangor 2006**).

A vaccination failure occurs when, following vaccine administration, the chickens do not develop adequate and are susceptible to a field disease outbreak. There are several factors, which cause vaccine failure including high level of maternal antibodies, stress of various types such as parasitism, environmental extremes and other concurrent disease can also contribute toward vaccine failure (**McMullin, 1985, Butcher and Miller, 2003, Shouq, 2004**). Health status of the birds is also important point of consideration as there may be incubating disease at the time of vaccination and time is needed for antibody production to begin and reach protective levels. The infectious agents such as infectious bursal disease, chicken anemia, Reo virus, Mycoplasma, coccidiosis and Salmonella cause varying degree of negative immunomodulation (immunosupression) which consequently led to vaccinal failure **(Butcher and Miller, 2003; Shouq, 2004, and Javid 2007**), also presence of mycotoxins in the feed affect the vaccinal response very badly.

Aflatoxins are a group of closely related toxic metabolites produced in feedstuffs by *Aspergillus flavus*. The most important of these toxins is aflatoxin B1. Research has examined the effect of aflatoxin on antibody responses and serum protein in chickens, the rational being that aflatoxin inhibits protein synthesis and therefore, impair antibody formation, **(Giambron et al., 1978).** Intoxication of birds by aflatoxin resulted in reduced complement activity, which is the most sensitive aspect of the immune system they alter, depression of cell – mediated immunity (CMI) and general immunosupression in chickens leading to poor vaccination response (**Thaxton et al., 1974) Giambrone et al., 1978, 1985, Ghosh et al., 1991 and Santin et al., 2001**). The concentrations of immunoglobulins Igm, IgG and IgA were reduced in chickens fed on diet contaminated with aflatoxins (**Giambrone et al., 1978**). The presence of low levels of aflatoxin B1 in the feed appears to decrease vaccinal immunity and may

therefore led to the occurrence of disease even in properly vaccinated flocks (**Lesson et al, 1995**). **Thaxton et al (1974)** recorded reduced antibody production following injection of sheep red blood cells in chickens experiencing aflatoxicosis. A study by **Gabal and Azzam (1998**) has described significant antibody decrease and mortality in young layer chicks vaccinated with commercial live attenuated vaccines against Newcastle disease, Infectious bronchitis and Infectious bursal disease following exposure to sub clinical dose (200 ppb) of aflatoxin in the feed. Several studies have shown that aflatoxin is immunosupression and its ingestion in feed has resulted in poor vaccination response (**Compel et al., 1988, Gush et al, 1990, Hegazi et al., 1991, Mohiudin, 1993; Azzam and Gabal, 1997). In a** simple term, **Thaxton et al (1974)** stated that immunosuprresive agents, the most commonly encountered, the aflatoxin may not allow the birds to achieve normal response vaccines.

Coccidiosis is one of the major parasitic disease affecting chickens and caused by Eimeria species acidosis cause significant mortality, high morbidity and adverse effect on the growth and feed potential of the infected chickens (**Akhtar and Ashaq, 2006)**. Eimeria species are regarded as ubiquitous parasites in most poultry environments, colonizing chicken guts after oral uptake of sporulated oocysts (**kabell et al., 2006)**. Beside the genetic factors playing a potential role in the final outcome of the coccidial infections, interferences of simultaneous infection with other pathogens such as viruses and bacteria can determine the severity of the disease **(Stephen and Barnett, 1964, Qin et al., 1995, Stroom and Sluis, 1999, Talukder et al., 2000, McDougald and HU, 2001)**. Concurrently, Caecal coccidiosis has been reported to be an immunosuppressive disease **(Paulos et al., 1997)**. It has also been experienced that whenever there is an outbreak of coccidiosis mainly caused by *Eimeria tanella* infection the vaccination failure occur and usually of the cases of Newcastle disease (ND, **Hegazay et al., 1986**) Infectious bursal disease (IBD; **Saha and Maumdar, 1997)** and Hydroperiardium syndrome (HPS, **Akhtar and Ashaq, 2006)** have been observed. It has shown been suggested that non-specific immunosupression caused by parasitic infection may make the host more susceptible to infections **(Bhanushali and Long.,1985)**. **Ros and Hesketh (1984)** have show that chickens infected with Eimeria tenella had suppressed to T- and B cell mitogens and have further suggested that infection with Eimeria may exacerbate heterologous infection with other species of Eimeria. **Anderson et al., (1977**) reported that Eimeria tenella mainly replicates in the epithelium of the cecae, but developing stages of E. tenella have been found in the bursa of fabricius. This finding may be

one of the exptanation of the immunosuppressive effect of E. tenella **(Bhopal et al., 1998**).

Mycoplasma gallisepticum is an economically important poultry pathogen causing respiratory disease in chickens and turkey **(Jordan, 1996, Ley and Yoder, 1997)** and it has immunosuppressive effect **(Ganapthy and Bradbury, 2003**). Also **Matsuo et al (1978) and Naylor et al., (1992)** reported possible immunosuppressive effect of *Mycoplasm gallisepticum* on a second infectious agents; **Matsuo et al (1978)** studied the suppressive effect of *Mycoplsama gallisepticum* (MG) on *Haemphilus gallinarum* (HG) immune response and found that humoral antibody response to HG was highly reduced and suppressed when chickens were inoculated with MG. Recovery rate and clinical symptoms of HG were more evident in chickens with suppression of antibody response (injected with mixture of MG & HG) than in chickens without suppression (injected with HG only). A similar finding was reported in dual infection of *M. gallisepticum* and avian pneumo-virus in turkey **(Naylor et al., 1992)** where a significant reduction was demonstrated on day 29 post– infection (p.i.) A recent study of **(Ouda et al.,2004**) investigated the effect of *Mycoplasma gallisepticum* infection on the efficacy of live Newcastle disease vaccine (NDV) in chickens and found that the homoral immune response (HI antibody liter) to NDV was significantly reduced in all birds infected with *Mycoplasma gallisepticum* either simultaneously or post NDV vaccination. These finding indicated that *Mycoplasma gallisepticum* may have immunosuppressive activity **(Matsuo et al.,1978).**Preliminary experiments have shown that other species of mycoplasma including *Mycoplasma arthritidis* and *Mycoplasma pneumonia* can suppress the serum antibody response of laboratory animals (**Kaklamans and Pavlatos,1972**). It has also been shown that *Mycoplasma meliagridis* and *Mycoplasma iowae* infection in turkey suppress the humoral immune response to non – replicating antigens **(Ortiz et al., 1981., Oritz and Yamamoto, 1981 and Bradbury, 1984).** Major infectious diseases of poultry have been controlled by immunization and effective management practices. Although such prophylactic measures are in place on most poultry farms outbreaks do occur. Lack of adequate protection and interference with immunity of birds seem to have important roles in such cases. Therfore this work was conducted to study the effect of dietary aflatoxin B1, *Eimeria tenella* and *Mycoplasma gallisepticum* on the huomral immune response to avian influenza inactivated vaccine in broiler chick**ens**

MATERIALS AND METHODS

1-Experimental chicks:

A total of two – hundred one – day – old broiler chicks, type (Hubbard) were obtained from a commercial hatchery on day of hatch. Chicks were housed in clean and disinfected separated pens and placed on a clean wood shaving litter at a temperature suitable for their age with continuous lighting and were reared under complete hygienic and management conditions. The chicks were allowed *ad libitum* access to feed and water for the entire experimental period. On arrival the chicks were tested to be mycoplasma- free, swabs for mycoplasma culture were taken from the choanal cleft of ten live chicks for attempted mycoplasma isolation **(Ganapathy and Bradbury, 1998).** All these samples proved to be negative for mycoplasma. According to vaccination program implanted in local broiler chicken farms, the chicks were vaccinated against Newcastle disease (ND), Infectious Bronchitis (IB) and Infectious Bursal Disease (IBD) using commercial vaccines as follow: at 5 days of age with Hitchner B1 Plus H 120 (for IBD) and with Lasota vaccine on day 21 of age. These vaccine were administered via eye drop instillation.

2-Feed:

The basal diets used in this study were unmedicated (without antibiotics and coccidiostat) commercial broiler rations which formulated to meet or exceded the recommended levels of nutrient requirements by National Research council **(National Research Council (NRC), 1994)** for broiler chickens. The birds were fed on a starter ration from 1 to 21 day of age then on grow diet for the period of 22 to 42 days of age. The feed was analyzed for the presence of mycotoxins according to **(Soares and Rodriguez – Amaya, 1989)** and found to be contain traces of ochratoxin (3.2 ppb) and free from any detectable other mycotoxins.

3-Avian influenza (AI) vaccine and vaccination procedure:

An inactivated avian influenza Type A H5N2 virus (A/chicken/ Mexico/ 232 – CPA/ 94) oil – emulsion vaccine obtained from local agency was used in this study.The experimental chicks were vaccinated again AI at seven day of the age, the birds received 0.5 ml of the vaccine / bird via subcutaneously injection in the

anterior dorsal cervical region as recommended by manufacturer and according to (Ellis et al., 2004).

4-Aflation:

a- Organism

Asperigillus parasiticus NRRL – 2999, a standard a flatoxogenic strain was used in this study for production of aflatoxin B1. The fungus was grown on potato-dextrose agar (PDA) at 28°C for 7 days to produce spores.

B. Aflatoxins production

Aflaxoxin was produced according to the method of **(Shotwell et al.,1966)** as modified by (West et al.,1973) by growing Asperigillus parasiticus NRRL – 2999 on sterilized crushed yellow corn (each 250 g) in 1000 ml conical flask, moistened by a 20 % (W/V) addition of sterile dist.Water and incubated in a dark place at 28°C for two weeks. The flasks were shaken once daily to reduce mycelial matting and prevent clumping the moldy corn was dried in an oven at 100°C for 12 hours to kill the fungus and then ground to a fine powder the corn powder then analyzed for determination of aflatoxin content by thin–layer chromatography (TLC) method according to the technique described by **(Soares and Rodriguez, Amaya, 1989)** and by HPLC method as described by **(Htchins and Hagler, 1983).**

C- Preparation of aflatoxin – contaminated diet:

Weighed amounts of the corn powder were mixed or incorporated into a standard commercial starter – grower diet which was analyzed & found not contain aflatoxin to provide a concentration of 2 mg aflatoxin / kg diet (2 ppm), the minimal growth inhibitory concentration of a flatoxin as described by **(Smith and Hamilton, 1970)**.

D- Induction of aflatoxicosis

A group of 40 broiler chicks was fed on aflatoxin contamination diet from one – day old till the end of experimental period, at 42 days of age to induce aflatoxicosis that proved and confirmed by histopathological examination.

5-Eimeria tenella

A- Eimeria strain

A pure field isolate of E. tenella strain isolated according to (Reley et al., 1976) from field outbreak of cecal coccidiosis that evidenced by prominent clinical signs, lesions and cecal smears was used in this study and maintained in our parasilogical lab.

B- preparation of E. tenella oocyst inoculm:

E. tenella oocysts was prepared for used as previously described by **(McDougald et al., 1997)**. The oocysts were processed for sporulation in 2.5% potassium dichromate solution **(Ryley et al., 1976**). Sporulated oocysts were given two washings with phosphate buffer saline (PBS, PH 7.2) the solution contained 2.00.000 (2×10^5) sporulated oocysts per ml as estimated by counting in a McMaster chamber under a microscope. Sporulaled oocysts were kept in a potassium dichromate solution (2.5%) at 4°C until use **(Akhtar and Ishaq, 2006)**

C- Determination of the dose

The dose of sporulated oocysts was adjusted to infect the birds and induce the disease without causing morality **(Williams, 2001, Kabell et al., 2006)**. The optimal dose was decided according to results of the following preliminary experiment similarly as described by **(Kabell et al., 2006)** Twelve one – day old broiler chicks were reared and vaccinated with routin vaccines acc to local vaccination program, when they were 15- day old divided into four groups of three chicks each and marked by leg marks. After feed withdrawal period of four hours sporulated *E.tenella* oocysts were given orally in the following doses, 0.10000, 15000, and 20000sporulated oocysts. The chicks were euthanized several days after inoculation, and autopathy was performed immediately to evaluate the degree of cocidial infection. Lesion scores results were evaluated according to **(Johnson and Reid, 1970)**, concerning *E. tenella*, 0 = no gross lesions, 1= Few scattered petechiae on the cecal wall, normal cecal wall somewhat thickened, blood present in Caecal contents, 3 = coalescent petechiae, cecal walls greatly thickened, much blood and fibrin in cecal contents, 4 = cecal walls greatly swollen and thickened, distended with blood or caseous clots, lesion score results were as follow: (000, 322, 333 and 443). From these result it was decided to inoculate two experimental groups with 20000 sporulated oocysts per bird.

D- E. Tenella inoculation:

Two groups of broiler chickens were orally inoculated with single dose of 20000 sporulated *E.tenella* oocysts/ bird in 1 ml volume, one at 14 days of age **(wyatt et al., 1975., McDogald and HU, 2001; Tammara et al., 2004)** and other group at 21 days of age **(Wyalt, et al 1975, Akhtar and Ishaq, 2006)**.Feed was withdrawn four hours before inoculation to facilitate the flow of oocysts into the gut. Then one ml of the sporulated oocysts stock solution was diluted in 9 ml tap water, and 1 ml of this diluted mixture was orally inoculated into each bird by using 2-ml long blunt end plastic pipette directly placed into the crop (McDogald and Hui 2001).

Detection of coccidial infection

At necrosy, the cecal lesion score were evaluated and scored 1 to 4 according to (**Jhonson and Reid, 1970)** as described under determination of the dose, depending on the thickness of cecal wall, cecal contents, and amounts of blood and caseous care, Also, infection was confirmed by histopathological examination of affected organs & tissues.

6-Mycoplasma gallisepticum(MG)

***A- Inoculum*:**

Mycoplasma gallisepticum (MG) S6 strain, a virulent reference strain of MG was used in the present study.The strain was kindly supplied by Dr. Abd – El – Ghany – Waffa, the Principal Investigator(PI) of the Research project " epidemiological, diagnostic and preventive studies on mycoplsama infection in breeder chickens", faculty of vet. Med. Cairo University. To prepare the inocula, as described by **(Bradbury, 1977, Jordan et al., 1991)**, the MG S6 strain was grown in Mycoplsama broth (MB). The broth culture was mixed gently, and was incubated at 37 C for 7 days in Co2 incubator. For determination of viable count, ten – fold serial dilutions of the broth – culture media were done then each dilution was inoculated on Mycoplasma agar (MA) plates. The MA plates were incubated at 37 C for 7 days in Co_2 incubator and count visible colonies. Titers were expressed as colony forming units (CFU) per milliliter (CFU/ml). The inoculum was checked for bacterial contamination by plating 20 ml of broth into blood agar **(Bradbury and Jordan, 1971)**.

B- MG – Experimental infection

On day 14 of age (i.e. 7 days post – AI vaccination) experimental mycoplasmosis was induced in a group of 40 broiler chicks according to **(Talkington and Keleven, 1985, Ganapathy and Bradbury 1998)**. Each bird was inoculated intransally with a dose of 0.4 ml of Mg S6 broth culture containing 2 x 10^5 cfu/ml (8 x 10^4 cfu/ chick). Birds in the control group were similar inoculated with 0.4 ml of sterile MB. The birds were observed daily for clinical signs and the infection was confirmed on the basis of histopathological examination.

7-Serum samples:

On arrival, ten randomly selected day – old chicks were slaughtered and blood samples were collected, then at weekly intervals from the 1st to 6th week of age ten birds from each group were bled via wing (ulnaris) veins and blood samples were taken. Blood sample were incubated for two hours at 37°C and then refrigerated overnight at 4°C. Sera were then separated by centrifugation at 3000 rpm for 10 minutes. The complement fractions of the sera were inactivated by heating serum samples in a water bath at 56°C for 30 min. **(Thaxton et al., 1974)**, then sera were stored at -20° C till tested.

Determination of humoral immune response to A.I Vaccine: Antibody titres produced passively (maternal) and actively in response to avian influenza inactivated vaccine were determined using standarad microhaemagglutination inhibition (HI) test procedure and ELISA techniqe

A-Haemagglutination (HA) and Haemagglutination inhibition (HI)test:

The recommended methods by **OIE (2005)** for HA and HI tests were applied in the sue of V-bottomed micro well plastic plates in which the final volume for both types of test (HA & HI) is 0.075 ml. The reagents required for these tests are isotonic phosphate Buffer saline (PBS) (0.01M and pH 7.0-7.2) and washed chicken red blood cells (RBCs). Positive and negative control antigens and antisera are required and should be run with each test, as appropriate

1- phosphate buffer saline (PBS):

Consists of sodium chloride (Na cl) 8.0g, potassium chloride (Kcl) water up to 1000 ml with pH (7.0 – 7.2).

2- Chicken red blood cells (RBCs) suspension:

Blood was collected from a minimum of 3-4 week – old chickens in sterile tubes containing 3.8% sodium citrate solution. An equal volume of phosphate buffer stain (PBS), pH 7.2 was added to the pooled blood samples, and RBCs were separated by centrifugation. The RBCs were washed with PBS three times. Before use, the RBCs were suspended in PBS as 1% concentration.

3- Reference antigen and antisera:

a) An avian influenza haemagglutinating antigen (A/ chicken / Mexi 323-CPA /94) $H5N_2$ antigen which represented the homologus antigen of the vaccine used in this study and was obtained from local agency and was used in HI.

b) Positive and negative avian influenza virus (AIV) antisera were obtained and were used in HI test.

Haemagglutination (HA) test procedure:

i) Despense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate. Place 0.025 ml of virus suspension (antigen) in the first well for accurate determination of the HA content, this should be done from a close range of an intial series of dilution, i.e 1/3, 1/4, 1/5, 1

ii) Despense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate.

iii) Place 0.025 ml of virus suspension (antigen) in the first well for accurate determination of the HA content, this should be done from a close range of an intial series of dilution, i.e 1/3, 1/4, 1/5, 1/6, etc.

iv) Make twofold dilutions of 0.025 ml volumes of the virus suspension across the plate.

v) Dispense a further 0.025 ml of PBS to each well.

vi) Dispense 0.025 ml of 1% (V/V) chicken RBCs to each well.

vii) Mix by tapping the plate gently and then allow the RBCs to settle for about 40 minutes at room temperature.

viii) HA is determined by tilling the plate and observing the presence or absence of tear – shaped streaming of the RBCs. The titration should be

read to the highest dilution giving complete HA (no streaming); this represent 1 HA unit (HAU) and can be calculated accurately from the initial range of dilutions.

Haemagglutination inhibition (HI) test procedure:

i) Dispense 0.025 ml of PBS into each well of a plastic V-bottomed plate.

ii) Place 0.025 ml of serum into the first well of the plate.

iii) Make twofold dilutions of 0.025 ml volumes of the serum across the plate.

iv) Add 4 HA units of virus / antigen in 0.025 ml to each well and leave for a minimum of 30 minutes at room temperature (back titration was done before every run).

v) Add 0.025 ml of 1% (V/V) chicken RBCs to each well and after gentle mixing, allow the RBCs to settle for about 40 minutes at room temperature, by which time control RBCs should be settled to a distinct button.

vi) The HI titer is the highest dilution of serum causing complete inhibition of 4 HA unites of antigen. The agglutination is assessed by tilting the plates. Only wells in which the RBCs stream at the same rate as control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) should be considered to show inhibition.

vii) The validity of results should be assessed a negative control serum, which should give a titre > 1/4 ($<2^2$ when expressed as reciprocal) and a positive control serum which the titre should be within one dilution the known titre.

HI titre may be regarded as being positive there is inhibition at a serum dilution of 1/10 (24 or log 4 when expressed as the reciprocal) more against 4 HA units of antigen.

B-Enzyme – linked immunosu – sorbant assay (ELISA):

A commercial ELISA kits was used for the detection of antibodies produced in response to avian influenza inactivated vaccine in each group. The protocol for ELISA was followed according to or (described in) manufacturer's instructions supplied with the kits. In brief, the reagents were allowed to keep at room

temperature and then mixed by inverting. The sample positions were recorded on a work sheet. Serum samples were diluted in 1: 500 dilutions with diluent buffer. An amount 100 µl of undiluted positive and negative controls and diluted samples were added to appropriate wells of ELISA plate (of the coated plates). Each sample and controls (positive and negative) was run in duplicate for optimum results. The plate was incubated at room temperature for 30 minutes then plates were washed 4 times with wash buffer (320 µ per well). An amount of 100 µl of enzyme conjugate reagent was added to each well and the plates were incubated for 30 minutes at room temperature. The plates were washed again as mentioned above. 100 µl of TMB substrate solution were dispensed into each well then plates incubated at room temperature for 15 minutes. An amount 100µl of stop solution was dispensed into (added to) each well and mixed by gently taping at the side of he plate to stop the reaction.The reading of the results including blank reader with air and the absorbance of the controls (positive & negative) and serum sample was taken and recorded by using a microtitre plate reader at 650- nm wavelength. The method of calculation for ELISA result was done according to prolocol supplied.

Calculation of ELISA results:

Results were expressed as the ratio between the optical density (OD) generated by the serum sample being tested (S) and the OD of positive control serum sample (P), i.e. the relative level of antibodies in tested sample was determined by calculating S/P ratio sample (S) to positive (P). The antibody $\log_{10}$ were calculated according to the equation provided with the kit:

$$\text{a) S/P ratio} = \frac{\text{optical densily (OD) of sample} - \text{OD of negative control}}{\text{OD of positive control} - \text{OD of negative control}}$$

b) titer

Log_{10} titer = 1.1 (Log_{10} S/P) + 3.156

Evaluation of growth performance

For determination of growth performance and feed efficiency per group, birds were individually weighed upon arrival and at day – 42 of age and feed consumption was re corded weekly. The body weight gain of chickens were

calculated by subtracting the live–body weight of chicks at one day old from live body weight of chickens at day 42 of age feed conversion rate (FCR) per group was determined at 42 days of a ge by dividing total feed consumption of chickens (gm) by total weight gain of chickens.

Histopathological examination:

It was performed in order to study the effects of aflatoxin, *E. tenella* and *Mycoplasma gallisepticum* on lymphoid organs and to correlate between these effect relevant to immune response to vaccination with AI inactivated vaccine (seroconversion). Also, histopathological examination was carried out to prove establishment of infection with *E. tenello* and mycoplasma as well as induction of aflatoxicosis. On day 42 of age, the study was terminated and five randomly selected birds per group were sacrificed and tissue samples from bursa of fabricius, thymus, spleen, ceacal tonsils, liver, air-sacs, cecum were taken. The tissues were fixed in 10% in 10% neutral buffered formalin. The fixed tissues were trimed ,embedded in paraffin ,sectioned at 4um and stained with hematoxylin and eosin **(Bancroft et al.,1996).**

Table (1): Experimental design: Vaccination against AI, Feed levels of aflatoxin, time of inoculation with E. tenella and Mycoplasma gallisepticum of various experimental groups of broiler chickens

Group number	Different Treatments				
	Vaccination at 7 day-old with AI vaccine	Aflatoxin in feed from 1 to 42 day-old (ppm – mg/kg)	Inoculation with E.tenella at 15-day ($2x10^3$ oocysts/bird)	Inoculation with E.tenella at 21-day ($2x10^3$ oocysts/bird)	Inoculation with M.gallisepticum at 15-day-old
A	No	Non	Non	Non	Non
B	Yes	Non	Non	Non	Non
C	Yes	Yes (2)	Non	Non	Non
D	Yes	Non	Yes (+)	Non	Non
E	Yes	Non	Non	Yes (+)	Non
F	Yes	Non	Non	Non	Yes (+)

Experimental design:

The experimental design is summarized and presented in table (1). On arrival the chicks were randomly into six equal groups (Group A, B, C,D, E, and F) each with 40 chicks and with two replicate pens of 20 chicks. The groups were housed separately and subsequently treated as follow: all birds were confined on aflatoxin

– free ration, while those in group C were fed on ration containing 2 ppm aflatoxin form 1-day-old till the 6 week of age. At the age of 7 days, chicks in all groups except that in group A were vaccinated with avian influenza inactivated vaccine. On day 14 of age, birds of group D were infected orally with *E. tenella* sporulated oocysts while bird in group F were intranasally infected with *Mycoplasma gallisepticum*.When birds in group E reached 21 day f age, they were infected with E. tenella sporulated ocysts via orall inoculation. Observation and registration included symptoms, pathology and serology. Three birds from toxicated and infected groups were euthanized 7 days - post infection and the carcases were subjected to post-mortem examination (autopathy) to prove the infection and toxication.

Statistical analysis:

Data for all response variables in the experiment were subjected to Analysis of Variance (ANOVA) **(Snedecor and Corchran 1980)** using INSTAT statistical software. Variable means for treatments showing significant difference in the ANOVA were compared using the Fisher's protected least significant difference (LSD) procedure. All statements of significance are based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Avian influenza has emerged as a disease with significant potential to disrupt commercial poultry production often resulting in extensive losses. Since 2003, a highly pathogenic form of avian influenza (H5N1 subtype) has devastated the poultry population in southeastern Asia, where it has already killed hundreds of millions of birds. H5N1 avian influenza has spread westwards and reached Middle East (Egypt), Europe and Africa in late 2005 – early 2006. The spread has affected more than 50 countries so far, which is unprecedented. The avian influenza H5N1 virus is responsible for hug economic losses, not only because of the high mortality rate induced but also because of the stamping policy. Vaccination of poultry against avian influenza is being recommended by international organization **(FAO and OIE 2004 and 2005**) and internationally recognized experts as a powerful and useful tool to control the disease and support eradication programs. Vaccination has been shown to increase resistance of birds to field challenge, reduce levels of viral shedding and reduce transmission and number of secondary outbreaks **(Capua 2007)**. All these effects of vaccination

contribute to control avian influenza and minimize the negative effects on animal welfare and potential economic losses in areas where the density of the poultry population would other result in uncontrollable spread without preemptive culling **(Capua and Marangon 2006).**

The rational behind the use of vaccination is that it should be able to generate a level of protective immunity in the target population. **(Capua 2007)**. Following vaccine administration, the ability of birds to mount an immune response and develop adequate and protective antibody levels can be reduced resulting in what is called vaccination failure. There are several factors which cause reduction in immune response to vaccination including infectious disease, stress of various type such as parasitism, concurrent diseases and mycotoxins **(Butcher and Miller, Javaed 2007).**

Health status of the flock is important point of consideration as there may be incubating disease at the time of vaccination and the bird become diseased at the time which is needed for antibody production to begin and reach protective levels **(Shouq 2004)**. The infectious agents such as chicken anemia, IBD, Mark's Reo, Salmonella, mycoplasma and coccidiosis cause varying degree negative immunomodulation which lead to vaccinal failure. Mycotoxins are another possible cause of poor immune response **(Javed 2007).**

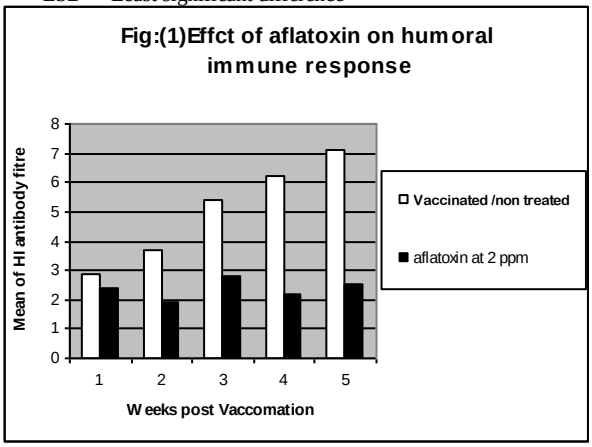
I. Evaluation of Humoral immune response:

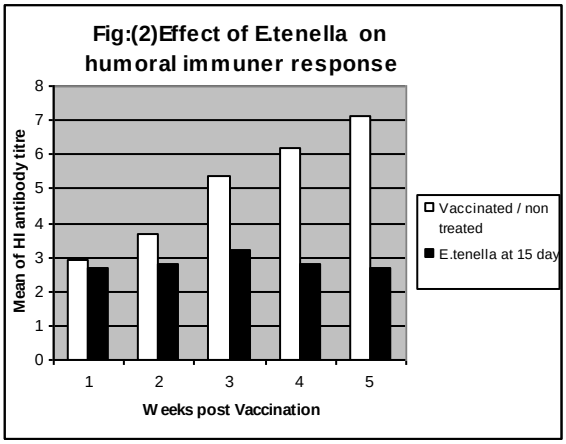
Data on the effects of dietary aflatoxin, *Eimeria tenella* and *Mycoplasma gallisepticum* infection on the antibody titers (both HI and ELISA antibody titers) at weekly intervals following vaccination of one – week broiler chicks with AI-inactivated vaccine are presented in table 2 and 3 & Fig. Data revealed that, the antibody titres were markedly and significantly ($P < 0.05$) higher in the vaccinated, untreated group (Positive control) than those of the unvaccinated, untreated group (negative control). The titres in the unvaccinated group seemed to be correlated to maternal immunity.

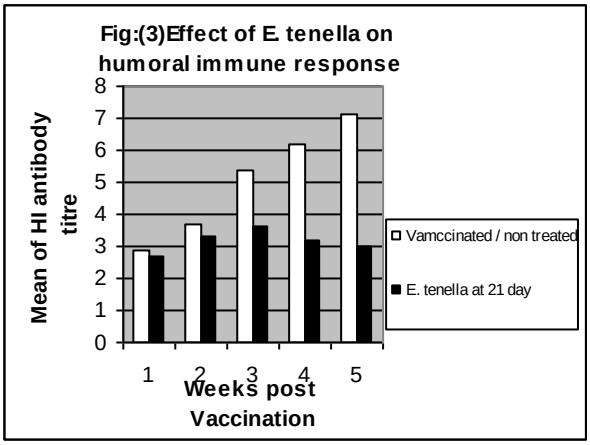
Table (2): Effect of dietary aflatoxin, *E. tenella* and *Mycoplasma gallisepticum* infection on himagglutinating inhibition (HI) antibody titers to avian influenza inactivated

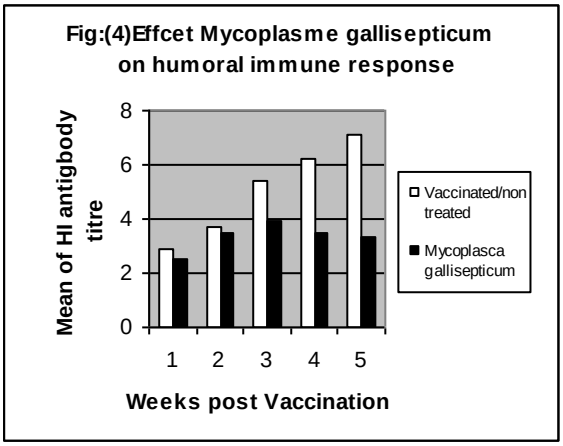
Experimental group		Mean of HI antibody titres (log_2) to AI inactivated vaccine						
No.	Treatment	Pre-vaccination		Post – vaccination				
		Age (Days)		Days post vaccination				
		1	7	7	14	21	28	35
A	Non- vaccinated-non-infected	5. 8±0.18	4.5±0.14	2. 6±0.14	3. 2±0.30	2. 4±0.28	1. 6±0.25	1. 2±0.34
B	Vaccinated- non-infected	5. 6±0.20	4.3±0.19	2. 9±0.19	3. 7±0.32	5. 4±0.44	6. 2±0.65	7. 1±0.32
C	Vaccinated + Aflatoxin	5. 2±0.29	4.1±0.20	2. 4±0.20	1. 9±0.15	2. 8±0.26	2. 2±0.16	2. 5±0.22
D	Vaccinated + E. tenella*	4. 7±0.24	4.3±0.29	2. 7±0.29	2. 8±0.24	3. 2±0.18	2. 8±0.30	2. 7±0.28
E	Vaccinated + E. tenella**	5. 5±0.33	4.4±0.29	2. 7±0.29	3..3±0.30	3. 6±0.26	3. 2±0.38	3. 0±0.32
F	Vaccinated + M.gallisepticum	5. 8±0.42	4.2±0.22	2. 5±0.22	3. 5±0.39	3. 9±0.41	3. 5±0. 34	3. 3±0. 26
	L.S.D	1.2	0.7	0.4	0.04	0. 91	1. 9	2.64

Values represent the Geometric mean ± SD of 2 riplicatesof 5 broiler chickens each per group.
Mean within the column with no common superscripts are significantly different. (P. < 0.05)
* Infected with E.tenella at 15- days of age ** Infected with E. tenella at 21- days of old.
LSD = Least significant difference









Effect of aflatoxin

A significant ($P < 0.05$) reductions in both haemagglulination inhibition (HI) and ELISA antibody titres to avian influenza vaccination did occur (Table 2 and 3) and Fig(1) in broiler chickens given dietary aflatoxin at dose level of 2.5 mg/kg feed. From hatching to 6 weeks of age when compared with the titres of birds in the vaccinated non–aflatoxin exposed group (group B) the reduction of antibody response was occured from as early as 15 day post – vaccination onward until the 42 days of age. Our results indicated that dietary aflatoxin at the level of 2 ppm is a potent immunosupression in broiler chickens.

Several alternative explanations for the immunosuppressive ability of aflatoxin exist. 1 aflatoxin has been demonstrated to inhibit RNA in vivo and subsequently to inhibit protein synthesis **(Lafarge and Frayssinet 1970).** Immunosupression by aflatoxin then would be the result of inhibition of the synthesis of specific immunoglobulin. 2. An enhanced degradation of antibodies would account for our results. Aflatoxin causes a rapid and dramatic increase in the specific activity of lysosomal enzymes in skeletal muscle and liver of chickens **(Tung et al., 1970)** and decrease in tissue strength and integrity **(Tung et al., 1971).** Since lysosomes and their hydrolytic enzymes are involved in the extracellular and intracellular digestin of macromolecules **(De Duve and Wattiaux, 1966)**, aflatoxin would be an immunosuppressant by vitue of its ability to stimulate lysosomal degradation of immunoglobulins. 3. Inhibition of the processing of antigen would also explain our results. Aflatoxin inhibits the reticuloendothelial system **(Michael et al ., 1973)**. These phagocytic cells possibly are involved in the processing of antigen **(Karnovsky , 1962)**. 4. Inhibition of specific immunological tissues is on attractive alternative explanation. The immunological system in chickens is dependent on the bursa of fabricius for initiating humorally–related anlibodier **(Glick, 1970)** and on the Thymus for initiating cellularly – related antibodies **(cooper et al., 1965)**. Regression of the cellular integrity of these tissues by chemical or physical agents results in immunosuppression **(Glick, 1976)**. Since the potential of lymphoid tissue to produce antibodies is dependent on the bursa and thymus the regression of the two organs by alfatoxin would be expected to result in impaired immunological performance and this is

supported by our histopathological findings in this study that revealed lymphocytic depletion in both bursa and thymus of broilers fed on aflatoxin contaminated diet.

Our results concerning with the effect of aflatoxin on humoral immune response to AI vaccination were in accordance with previous finding by **(Azzam and Gabal 1998**., **Gabal and Azzam, 1998**)) who studied the impact of aflatoxin in the feed on the prophylactic vaccination (immunization) against Newcastle disease, Infectious Bronchitis Infectious Bursal Diseas and Fowl Cholera in both young layer chicks and layer hens.They found that ingestion of aflatoxin contaminated feed significantly reduced antibody titres compared to non-aflatoxin treated groups. Also our study agree with that of **(Shivachandra et al., 2003; Otim et al 2005)** who found a significant ($P < 0.05$) reduction in the haemagglutination inhibition of ND antibody titres following initial priming with Hitchner B1 at 21 day of age and booster with La Sota vaccines 3 week later in broiler chicks injected intramuscularly at the age of 3 weeks every 2 days up to four times with 0.250 mg afltoxin B1 per bird. Brioler chickens fed on diet contaminated with subclinical levels of aflatoxin from 10 days of age and continued for 8 weeks and vaccinated against IBD resulted in significant decrease in antibody response to IBD vaccination **(Azzam and Gabal, 1997)**.

This indicated that aflatoxin in the feed over a protracted period of time result in serious adverse effects on the immune system. Other studies have shown that aflatoxin is immunosuppressive and its presence in feed and ingestion has resulted in decreased immunity in vaccinated birds and may therefore lead to the occurrence of disease even in properly vaccinated flocks **(compbell et al., 1998; Gush et al., 1990; Hegazi et al; 1991; Mohiudin, 1993; lesson et al., 1995)**. Aflatoxin at dietary level of 1 mg/kg feed (1 ppm) or more caused a reduction in humoral immune response determining by the antibody response to sheep red blood cells (SRBC) **(Thaxton 1974; Viride et al., 1989; Verma et al., 2004; Singh et al., 2006)** recorded significant reductions in haemagglutinating (HA) antibody

titres against SRBCs in broiler chickens experiencing aflatoxicosis. **Batra et al., (1991)** found that chickens fed aflatoxin B1 and vaccinated against Marek's disease showed a significantly higher frequency of gross and microscopical lesions of Marek's disease than did chickens fed aflatoxin free diet.

Table (3): Effect of dietary aflatoxin, *E.tenella* and *Mycoplasma gallisepticum* infection on the ELISA antibody titres to Avian influenza inactivated vaccine.

Experimental group		Mean of ELISA antibody titers (log_{10}) to AI inactivated vaccine						
No.	Treatment	Pre-vaccination		Post – vaccination				
		Age(Days)		Days post vaccination				
		1	7	7	14	21	28	35
A	Non- vaccinated-non-infected	4. 11	3.95	3. 22	3.10	3. 08	2. 92	2.73
B	Vaccinated- non-infected	3. 85	3.65	3. 44	4. 66	5. 47	5. 36	5. 41
C	Vaccinated + Aflatoxin	3. 66	3.44	3. 21	2. 81	2. 45	2. 40	2. 21
D	Vaccinated + E. tenella*	4. 14	3.82	3. 64	3. 04	2. 81	2. 56	2. 39
E	Vaccinated + E. tenella**	3. 78	3.59	3. 15	3. 35	3. 10	2. 79	2. 18
F	Vaccinated + M. gallisepticum	3. 81	3.74	3. 61	3. 44	3. 21	2. 33	2. 23

Values represent mean of ELISAantibody titres (log_{10}) to AI inactivated vac
* Infected with E.tenella at 15- days of age ** Infected with E. tenella at 21- days of old.
LSD = Least significant difference

Effect of Eimeria tenella infection:

Antibody production with time after vaccination with AI vaccine in broiler chickens experimentally induced with ceacal coccidiosis are shown in Table (2 & 3)and Fig(2-3) . Haemagglutination inhibition (HI) antibody titres and ELISA titres against AI vaccine were to found decreased significantly ($P < 0.05$) in birds experimentally infected with E.tenella at 21 days of age (group E) and highly significant ($P < 0.01$) in those infected with *Eimeria tenella* at 15 days of age (group D) when compared with vaccinated, non-infected birds (group B).

The significant reduction in antibody titres that was recorded in group (IV) was more than that observed in group (V). This significant difference in titres between birds experimentally infected with *E.tenella* at 15 days of age and those infected at 21 day of age may be due to the infection at 15 days of age (7 days post AI vaccination) was the needed for antibody production to begin and onward reach to protective levels **(Shouq 2007)**. This data indicated that infection of broiler chicks with *E.tenell* infection resulted in poor humoral immune response to AI vaccine. Similar findings have been reported on Newcastle disease (ND) and Infectious bursal disease (IBD) that the antibody titres against these diseases significantly

decreased up to such extent that outbreaks of occurred in the vaccinated chickens and experimentally induced with ceacal coccidiosis **(Mohammed, 1980; Hegazy et al., 1986; Wanis et al., 1991, 1991a; Okay, 1993; Oraby et al., 1994; Saha and Majumdar, 1997; Talukder et al., 2000)**. Also **Ashfaq and Akhtar (2006)** reported that broiler chicks experimentally infected with mixed species of Eimeria species including E.tenella, E. necatrix, E. acerrulina and E. maxima and vaccinate against hydropericardium syndrome (HPS) had significant reduced HI antibody titres than non-infected vaccinated birds.

Such a low level of antibody titres against AI vaccination in broiler chickens experimentally induced with caecal coccidiosis may be due to the immunosuppressive effects of the disease **(Man et al., 1971; Paulos et al., 1997; Bhopal et al., 1998)** which cause immune deficiency due to destruction of lymphoid tissues in bursa of fabricius and thymus that lead to reduce response to routine vaccination **(Shatshneide and Perri, 1976; Khovanskikh, 1978; Olariu-Jurca et al., 1994, 1997).** Other possibility exist to explain the mechanism by which caecal coccidiosis reduce the humoral immune response is the stress exerts by the parasite on the birds The stress resulted in rising in the blood level of corticosteroid **(Siegel, 1980., Dohms and Metz, 1991)** corticosteroid are thought to act by influencing the rate of synthesis of specific RNA and protein, resulting in lymphopena and lymphoid cell destruction (atrophy of lymphoid organs) and subsequently reduced antibody production (Thompson and Lippman, 1974 ; Gould and Siegel, 1980; Dohms and Metz, 1991).

Effect of Mycoplasma gallisepticum:

Serological pattern of broiler chickens experimentally infected with *Mycoplasma gallisepticum* following vaccination with avian influenza inactivated vaccine was assessed. The results revealed a significant ($P > 0.05$) decrease in antibody titres to AI vaccine (measured by HI and ELISA tests) when compared with non-infected vaccinated group, (Table 2 and 3).and Fig(4) This result indicated that *Mycoplasma gollisepticum* had a negative impact on the humoral immune response of broiler chickens evidence by poor immune response (low HI and ELISA antibody litres) to AI inactivated vaccine.

The fact that there was suppression of antibody production to AI vaccine after infection with *Mycoplasma gallisepticum* is supported by some earlier

observations with Mycoplasm gallisepticum. For example **(Matsuo et al., 1978)** have shown that in a mixed infection with *Mycoplama gollisepticum and Haemophilus gallinarum*, there was a reduction in the humoral antibody response to the other organism. A similar finding was reported in dual infection of *Mycoplasma gallisepticum* and avian pneumovirus in Turkey **(Naylor et al., 1992).** According to these finding **(Matsuo et al., 1978 and Naylor et al., 1992)** indicated that *Mycoplasma gallisepticum* may have a possible immunosuppressive activity. The immunosuppressive effect of *Mycoplasma gallisepticum* in broiler chicken was confirmed by **(Quda et al., 2004)**, They studies the effect of MG infection on the immune response to ND vaccination in broiler chickens and found a significant decrease in immune response to ND vaccines expressed by low HI antibody titres in all groups infected with *Mycoplasma gallisepticum* simultaneously with or one week post ND vaccination compared to control group.

Our results are consistent with the previously reported by in parallel study o (Matsuo et al., 1978) who found that antibody response to *Haemophillus gallinarum* was highly suppressed when chicken were inoculate with *Mycoplasma gallisepticum* indicating the immunosuppressive effect of *Mycoplsama gallisepticum*. Infection of young turkey with *Mycoplasma iowae* resulted in a significant reduction in the humoral immune response to sheep red blood cells (SRBC) and in bural/body weight ratio **(Bradbur 1984)** and he concluded that *Mycoplasma iowae* may cause temporary damage to the bursal of fabricicus accompanied by a transient immunosuppression. **(Ortize et al., 1981)** found that *Mycoplasma meliagridis* (MM) infected turkey significantly lower antibody titre against primary and secondary antigenic stimulation with inactivated *Salomenell pullorum* or dinitrophenyl- bovine gamma globulin. They concluded that the immune response in turkeys infected with *M. Meliagridis* is similar to that of bursectomized chickens. A significant decrease in HI antibody titres to inactivated ND vaccine was recorded in young turkey infected with *Mycoplasma meliagridis* when compared with vaccinated non-infected turkey **(Ortize and Yamamoto, 1981)**

The mechanism whereby *Mycoplasm gallisepticum* exerts its immunosuppressive effect on humoral immune response can not be stated on the basis of the present stud, but there are several possibilities exist to explain the mechanism. Most probable possibilities are 1-, Mycoplasma infection induced a stress on the bird and the stress in turn induce immunosuppressive. The commn pathway for stressors involves the hypothalamic-pitutiary-adrenal(HPA)axis and result in release and increaseblood level of glucocorticoids which are immunosuppressive for many specie including chickens **(Siegle, 1980)**, **Dohms and Metz, 1991)**. Corticosteroids are though to act by influencing the rapid atrophy and destruction of lymphoid cells (rapid lymphoid depletion) in bursa of fabricius, thymus and spleen and subsequently reduced antibody production **(Thompson and Lippman, 1974; Gould and Siegel, 1980; Dohms and Metz 1991)**. These authors suggested that bacterial infection (e.g. *Escherichia coli*) may cause stress-type lesions in the bursa similar to corticosteron – induced lymphoid depletion.An alternative possibility is that mycoplasma interferes with the antigenic processing by macrophage.

Evaluation of performance

Final body weight, body weight gain and feed conversion ratio of the different group are presented in table (4) . As can be seen fro the table, no significant difference between the non-vaccinated, non-infected group (group A) and vaccinated, non- infected group (group B) in relation to performance data indicating that avian influenza inactivated vaccine on the performance of broiler chickens. The body weight, body weight gain and feed efficiency of broilers in group C, D, E and F were significantly ($P< 0.05$) decreased compared to those in vaccinated, dietary aflatoxin, *Eimeria Tenella* and *Mycoplasma gallisepticu* infection had a negative impact on performance of broiler chickens with dietary afilatoxin in higher levels in experimental conditions resulted in significant reduction in body weight, weight gain and impaired feed efficiency.

Table (4): Effect of dietary aflatoxin, *Eimeria tenella* and *Mycoplasma gallisepticum* on body weight body, wt gain and feed conversion ratio (FCR) on day – 42 of age

Experimental group	Mean ± SD of initial body weight, Final body wt, Body wt. gain & Feed conversion (FCR)			
No. Treatment	Initial body wt at 1-day-old	Final Body wt on day 42 of age	Body wt gain on day 42 of age	Feed conversion ratio (FCR)
A Non- vaccinated-non-infected	46.70± 1.90	1805. 80±36.55	1758. 55±28.52	1.75
B Vaccinated- non-infected	45.48± 1.88	1786..26±45.18	1741.14±32.44	1.84
CVaccinated+ Aflatoxin	45.38± 1.72	1319.83±25.43	1274.45±24.50	2.47
D Vaccinated + E. tenella*	47.45± 1.80	1389.50±51.13	1342.83±30.41	2.33
E Vaccinated + E. tenella**	46.57± 1.47	1513.45±38.19	1466.83±27.14	2.40
F Vaccinated + M.gallisepticum	45.65± 1.67	1622. 65±26..55	1576.78±38.19	2.15
L.S.D		**164.60**	**141.25**	

- Values represent Mean ± SD of thirty broiler chickens per group (n = 30)
- Mean bearing common superscripts in individual column did not differ significantly (P < 0.05)
- * Infected with E.tenella at 15 days of age** infected with E.tenella at 21 days of age
- LSD mean least significant diff

Also the results obtained herein in relation to effect of *Eimeria tenella* on performance are in accordance with those of **(Mathis et al., 2003, 2004, Lee et al., 2007a and 2007b)** who indicated that **Eimeria tenella** infection caused significant adverse effects on the growth and feed potentials of the infected broiler chickens. Concerning with *Mycoplsama gollisepticum* the results of the present study are in agreement with the finding of **(Kleven, 1998, Kempf et al., 1998., Jordan,1996)** who concluded that *Mycoplsama gallisepticum* infection in chickens with or without complicating pathogen causes poor growth and reduced feed efficiency

Histopathological results:

The histopathological alteration occurred in different organs obtained from different experimental groups presented in the figures 5,6,7,8 and 9. The results of histopathological examination supported and confirmed the serological results obtained in the present study as Aflatoxins, *Eimeria tenella* , and *Mycoplasma gallisepticum* resulted in lymphocytic depletion in the lymphoid cells of bursa of fabricius spleen and thymus. This lymphocytic depletion resulted in reduction in the humoral immune response against avian influenza vaccine in the broiler chickens kept on these treatments. Also the histopathological results as showen in Fig. (6,7and9) proved the pathogenicity of *E. tenella* and *Mycoplasma gallisepticum* strains used in this study and stablishment of the infection with both as well as the histopathological alterations observed in aflatoxin-treated group confirmed the induction of aflatoxicosis in this group Fig, (5).

In conclusion this study indicated that dietary aflatoxin ,Eimeria tenella and *Mycoplasma gallisepticum* caused negative immunomodulation and poor immune response to avian influenza inactivated vaccine in broiler chickens. This adverse effect on the humoral immune response may result in occurring of avian influenza

REFERENCES

Akhtar, M., Ishaq, H. M. (2006): Effect of coccidiosis on the humoral response of chickens vaccinated against hydropericardium syndrome. Journal of veterinary Research, 10: 1-6.

Anderson, W. I., Reid, W. M., Lukert, P.D. and Fletcher, O.J.J.R. (1977) Influence of Infectious bursal disease on the development of immunity to Eimeria tenella. Avian Disease, 21: 637-641.

Azzam, A. D. and Gabal, M.A. (1997): Interaction of aflatoxin in the feed and immunization against selected infectious diseases. I. Infectious bursal disease. Avian pathology, 26: 317-325.

Azzam, A. H. and Gabl, M. A. (1998): Aflatoxin and immunity in layer hens. Avian pathology, 27: 570-577.

Banchroft, J. D.; A. Stevens, and D. R. Turner (1996): Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, NewYork, London, San Francisco, Tokyo

Batra, P., Pruthi, A. K., and Sadana, J. R. (1991): Effect of AFB1 of the efficacy of turkey herpes virus vaccine against Marek's disease. Res. Vet. Sci., 51: 115-119.

Bhanushali, J. K. and long, B. L. (1985): Eimeria tenella infection. Does it affect humoral immune response to heterologous antigens. Journal of parasitology, 71: 397-409.

Bhopal, S. T., Deore, M. D., Gathne, M. L. and Naraspur, N. (1998): Immunosuppress in birds experimentally infected with single and mixed parasitic infections. J. Bombay Veterinary college, 6: 17-19.

Bradbury, J. M. (1977): Rapid biochemical tests for characterization of the Mycoplasmatales, Journal of clinical Microbiology, 5: 531-534.

Bradbury, J. M. (1984): Effect of Mycoplasma iowae infection on the immune system of the young turkey. Isr. J. Med. Sci., 20: 985-988.

Bradbury, J. M. and Joran, F. T. W. (1971): The influence of PH of the culture medium on the sensitivity of Mycoplasma gallisepticum antigens for use in certain serological tests. Journal of Hygiene, 69; 593-606.

Butcher G. D., and Miller, R. D. (2003): Vaccine failure in poultry: factors to consider. Florida cooperative Extension service Institute of food and Agricultural science, University of.

Calnek, B.C., Barnes, H. J., McDougald, L. R. and Saif, Y. M. (1997): Disease of poultry 10th ed, pp. 951-979. Moslaby Wolfe, Iowa Stale Univ. Press, Ames, Iowa, USA.

Campbell, M. L., May, D., Huff, W. E. and Doer, J. A., (1988) Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. Poultry Sci., 62: 2138-2144.

Capua, I. and Alexander D. J. (2004): Avian influenza recent developments. Avian pathology, 33: 394 – 404.

Capua, I., Cattoli G., Marangon S., DIVA. (2004): Avaccination strategy enabling the detection of field exposure to avian influnenza. Dev Biol., 119: 229 – 33.

Capua, I and Marangon, S. (2006): Control of avian influenza in poultry. Emerging infectious Disease (EID) Journal, 12: 1-15.

Capua, I. (2007): Vaccination for notifiable avian influenza in poultry. Rev. Sci. tech. off. Int. Epiz., 26 (1), 217-227

Cooper, M. D., Peterson, R.D.A., South, M. A. and Good, R. A. (1965): The functions of the thymus system and bursa system in the chicken. J. Exp. Med., 123: 75-123.

DeDuve, C., and Wattiaux, R. (1966): Functions of lysosomes. Amm. Rev. physiol, 28: 435-492.

Dohms, J. E. and Metz, A. (1991): Stress mechanisms of immunosuppression. Avian Disease, 28: 305-310.

Ellis, T. M., Leung, C. Y. H., Chow, M. K. W., Bissett, L. A., William, W., Yi, G. and Malik Peris, J. S. (2004): Vaccination of chickens against H5N1 avian influenza in the face of an outbreak interrupts virus transmission. Avian pathology, 32: 405 – 412.

Gabal, M. A., and Azzam, A. H. (1998): Interaction of aflatoxin in the feed and immunization against selected infectious diseases in poultry. II. Effect on one – day- old layer chicks simultaneously vaccinated against Newcastle

disease, Infectious bronchitis and Infectious bursal disease. Avian Pathology, 27: 290.

Ganapathy, K., and Bradbury, J. M. (1998): Pathogenicity of Mycoplsama gallisepticum and Mycoplsama imitans in red – legged partidges (Alectoris rufa) Avian pathology, 27: 455-463.

Ganpathy, K, and Bradburg J. M. (2003); Effect of cyclosporin A on the immune responses and pathogenesis of a virulent strain of Mycoplasma gallisepticum in chickens. Avian pathology, 32: 495 – 502.

Ghosh, R. C. Chaughan, H.V.S. and Jhe. G.J. (1991): Suppression of cell mediated immunity by aflatoxin B1 in broiler chicks. Veterinary immunology and Immunopathology, 28, 165 – 172.

Giambrone. J. J., Ewert D. L. Wyalt R. D. and Eidson C. S. (1978): Effect of aflatoxin on the humoral and cell-mediated immune system of the chickens. Am. J. vet. Res., 39: 305 – 307.

Giamborn, J. J., Deiner, U.L., Davis, N. D., Pargala, V. S. and Hoerr, F. J. (1985): Effect of aflatoxin on broiler chicken. Poultry Sci., 64: 852-858.

Glick, B. (1967): Antibody and gland studies in cortisone and ACTH – injected birds. J. Immune.

Glick, B. (1970). The bursa of fabricius as a central issue. Biosccience, 20: 10-13.

Gould, N. R. and Siegel, H. S. (1980): Effect of adrenocorticotropin on binding of endogenous corticosteroid by chicken bursa cells. Poultry Scd., 59, 1935-1940.

Gush, R. C., CHauhan, H. V., and Roy, S. (1990): Immunosuppression of broiler under experimental aflatoxicosis. British veterinary Journal, 146: 457-462.

Hatchins, J. E. and Hagler, W. M. (1983): Rapid liquid chromatographic determination of aflatoxin in heavily contaminated corn. Journal of official analytical chemistry, 66: 1458-1465.

Hegazy, N. M., Nada, M. S. and Gowady, H. E. (1986): Eimeria tenella infection in chickens and immune response to Newcastle disease vaccine. Assiut Veterinary Medical Journal, 17: 195-197.

Hegazi, S., Azzam, A. H. and Gabal, M. A. (1991): Interaction of naturally occurring aflatoxin in the feed and immunization against fowl cholera. Poultry Science, 70: 2425-2428.

Huff, W. E., Kubena, L. F., Harvey, R. B., correir D. E. and Mollenhauer, H. H. (1986): Progression of aflatoxicosis in broiler chickens. Poultry science, 65: 1981-1999.

International Food Safety Authorities Network (INFOSAN), (2005): Avian influenza, INFOSAN information Note No. 7/ 2005.

Javed T., (2007): Causes and effects of vaccination failure. Pakastan. Com.

Johnson, J. and Reid, W. M. (1970). Anticoccidial drugs. Lesion scoring technquis in battery and floor pen experiments with chickens. Exp. Parasitol, 28: 30-36.

Jones, F.T., Hagler, W. H. and Hamilton, P.B. (1982): Association of low levels of aflatoxins in feed with productivity losses in commercial broiler operations. Poultry science, 16: 861 – 868.

Jordan, F. T., Bradbury, J. M. and kleven S. H. (1991); Mycoplasmosis (Mycoplasma gallisepticum) Manual of recommended diagnostic techniques and recommendations for biological products. Volum (3). Office International Des Epizotices (OIE), Paris.

Jordan, F. T.W (1996): Avian mycoplasmas. In Jordan, F.T.W and patission, M. (eds), poultry diseases, 4th edn (pp.81 – 93) London: W.B. saunders company ltd.

Kabell, S., Hamdberg, K. J. and Magne Bisaard, (2006): Impact of coccidial infection on vaccine-and vv IBDV in lymphoid tissues of SPF chickens as detected by RT – PCR. Acta Veterinaria Scandinavica, 48: 17-22.

Kaklamanis, E. and Pavlatos, M. (1972): The immunosuppressive effect of Mycoplasma infection. I. Effect on the humoral and cellular response. Immunology, 22: 695-702.

Karnovsk, M. L. (1962): Metabolic basis of phagocytic activity. Physiol. Rev., 42: 143-165.

Kempf, I., van den hoven, R., Gesbert, F. and guittet, M. (1998): Efficacy of difloxacin in growing broiler chickens for the control of infection due to pathogenic mycoplasma gallisepticum. Zentrabl veterinarmed B., 45 : 305 – 310.

Khovanskikh, A. E. (1978): The mechanism of depression of the immune system in chicken affected with coccidiosis. Proceedings of the 4th International congress of Parazitologicheskie Issledovaniya V. Pridltike. 61–64.

Khovanskikh, A. E. (1978); The mechanism of depression of the immune system in chicken affected with coccidiosis. Proceedings of the 4th international congress of parasitology, Warsaw, Poland. 65.

Kleven, S.H. (1998): Mycomplasmas in the etiology of multifactor ial respiratory disease. Poultry science, 77 : 1176 – 1149.

Lafarage, C., and Frayssinet (1970): The reversibility of inhibition of RNA and DNA synthesis induced by afaltoxin in rat liver. Int. J. Cancer, 6: 74-83.

Lee, S., Lillehoj, H.S., Park, D.W., Hong, Y.H. and Lin, J.J. (2007a): Effects of pediococcus and saccharomyces based probiotic (mito max) on coccidiosis in broiler chickens. Comp Immunol. Microbial. Infect. Dis., 30: 261 – 268.

Lee, S.H., Lillehoj, H.S. Dolloul, R.A. Park , D.W., Hong, Y.H. and lin, J.J. (2007b): Influence of pediococcus based probiotic on coccidiosis in broiler chickens. Poultry science, 86: 63 – 66.

Lesson, S., Diaz, G. J. and Summers J. D. (1995): Poultry metabolic disorder and mycotoxins PP 249-298, university Books, Guelph, antrario, Canada.

Ley, D. H and Yoder, Jr., H. W. (1997): Mycoplasma gallisepticum infection. In B.W. Calnek, H. J. Barnes, C. W. Bear L. R. McDougald and Y. M. Saif (Eds). Disease of poultry, 9th edn (pp. 191- 207). Ames, IA: Iowa state university Press.

Man, C., Angy, E. and Mldovan, N. (1971): Stress induced in chicks by experimental infection with Eimeria tenella. Seri Medical Veterinary, 27: 331-339.

Mathis, G.F.,Frooyman,R.,Irion,T.and Kennedy,T.(2003): Coccidiosis control with toltrazuril in conjunction with anti coccidial medicated or nonmedicated feed .Avian DISEASE, 47:463-464

Matsuo, K., Kuniyasu, C., Yamada, S., Susuni, S., and Yamamoto, S. (1978): Suppression of immunoresponses to Haemophilus gallinarum with nonviable Mycoplasma gallisepticum in chickens. Avian disease 22: 552 – 56.

McDougald, L. R., Fuller, L. and Mattiello, R. (1997): A survey of coccidia on 43 poultry farms in Argentina. Avian Dis., 41: 923-929.

McDougald, L. R. and Hu, J. (2001). Blackhead disease (Histomonas meligridis) Aggravated in broiler chickens by concurrent infection with caecal coccidiosis (Eimeria tenella). Avian Disease, 45: 307-312

Mc Mullin, P. (1985): Factors which interfere with vaccine efficacy proceeding of the 1st sta. calerina poultry symposium pp 10-20.

Michael, G. Y., Thaxton, J. P. and Homilto, P.B. (1973): Impairment of the reticuloendothlial systems of chickens during aflatoxicosis. Poultry science, 52: 1206 – 1207.

Mohammed, M. A. (1980): Effect of subclinical infection with Eimeria tenella on the response of chickens to vaccination against Newcastle disease. Rechive Fur Gflugel Kunde, 44: 47-49.

Mohiudin, S. M. (1993): Effects of aflatoxin on immune response in viral diseases. Poultry Adviser, 26: 63-66.

National Research Council(NRC) (1994): National Requirement for poultry, 9th edn (Washington DC, National Acadmy Press)

OIE (office international des Epizooties), FAO (Food and Agricultura organization) (2005): Recommendation of the second FAO/OIE regional meeting on avian influenza control in Asia. Ho chi Minh city 23-25 February 2005.

OIE (office International des Epizooties), FAO (Food and Agriculture Organization) (2004): Recommendations of the FAO/ OIE emergency regional meeting on avian influenza control in Asia, Rone, 3-4 February 2004, and Bongkok 26-28 February 2004.

OIE international des Epizooties) Manual (2005): OIE manual for diagnostic tests and vaccines for terrestrial animals. Chapter 2.7. 12., Version adopted May, 2005.

Naylor, C. J., Al-Ankari, A. R., Al-Afaleq, A. I., Bradbury, J. 1 and Jones, R. C. (1992): Exacerbation of Mycoplasma gallisepticum infection in turkey by Rhinotracheitis virus. Avian pathology, 21: 295-305.

Okoy, J. (1983): The effect of late infectious bursal disease on the severity of naturally occurring Eimeria necatrix infection in chickens. Bulletin Animal Health and Reproduction, Africa, 31: 263-267

Olariu- Jurea, I., Coman, M., Manu, G. and Falea, C. (1994): Microscopic histological and haematolgical studies of chickens with coccidiosis. Lucrari Strintifice. 28: 53-56.

Olariu – Jurca, I., Coman M., Manu. G., Darabus, G. Oprescu, I, Morariu, S., Serdean, C., Cearnau, I. and Cumpana. C. S. (1997): Morph-Pathological aspect of caecal Eimeriosis in the hen. Lucrari stintific. 31: 231 – 235

Oraby, F. A., Edris, G. O., Hegazy, A. G. Aggour, M. G. (1994): Possible immunosuppressive effect of Eimeria greniere infected guinea fowl: the effect of vaccination with Newcastle Disease virus (k strain) Ann. Agriculture Science, 32: 2205-2211.

Ortiz, A. M. and Yamamoto, R. (1981): Suppression of the humoral immune response to inactivated. New castle disease virus by Mycoplasma meligridis in the turkey Avian Disease, 25: 894- 899.

Ortiz, A. M., Yamamoto, R., Benedict, A. A. and Mateos, A. P. (1981): The immunosuppressive effect of Mycoplosma meliagridis on nonreplicating antigens. Avian Disease, 25: 945- 963.

Otim, M. O. Mukib- Muka, G., Christenen, H. and Bisgaard, M. (2005): Aflatoxicosis, infectious bursal disease and immune response to Newcastle disease vaccination in rural chickens. Avian pathology, 34: 319-323.

Ouda E. Sahar, Hussein, H. A., Laila, M. El-shabiny, and Shalaby, M. A. (2004): The effectof Mycoplasma gallisepticum infection on New castle Disease virus vaccination efficacy, proceeding of 6th Scientific conference of the Egyptian Veterinary poultry Association, September 25th – 27th 2004 (pp., 342 – 359).

Paulos, C., Cruz-Te, D. and Cruz- Te, E. (1997): Immunodepression as the cause of the main failures (with cases of caeca coccidiosis) in broiler production. Veterinaria Tecnica, 7: 40-49.

Qin Z. R., Arakawa, A., Baba, E., Futkata, T., Miyamoto T. and Sasai, K. (1995): Eimeria tenella infection induces recrudescence of previous salmonella entritidis infection in chickens. Poultry science, 74: 1786-1792.

Reddy, D.N., Roo, P.V., Reddy, V.R and Yadgiri, B. (1984): Effect of selected levels of dietary aflatoxin on the performance of broilers chickens. Indian J. avian science, 54: 68 – 73.

Rose, L. H., and Hesketh, S. B. (1984): Journal of Protozoology, 31: 549-552.

Ryley, J. F., Meade, R., Ifazalburst, J. and Robinson, T. E. (1976): Methods in coccidiosis research: separation of oocysts from faeces. Parasitology, 37: 311-326.

Saha, A. K. and Majumdar, A. K. (1997): Report of infectious bursal disease among chicken in Tripura. Indian veterinary Journal, 74: 515-516.

Santin, E., Maiorka, A., Macari, M. Grecco, M., Sanchez, J. C., Okada, T. M. and Myasaka, A. M. (2001): Performance and intestinal mucosa development in broiler chickens fed ration containing Saccharomyces cerevisiae cell wall. Journal of Applied poultry Research, 10: 236-244.

Shatshneider, T. and Parri, Y. U. (1976): Changes in the cell structure of lymphoid organs during ceacal coccidiosis of chicks. Parazitologicheskie Issledovaniya V. Pridltike. 61-64.

Shivachanda, S. B. Sah, R. L., Singh, S. D, Kataria, J. M. and Manimaran, K. (2003): Immunosuppression in broiler chicks fed aflatoxin and inoculated with fowl adenovirus serotype-4 (FAV-4) associated with hydroperioardium syndrome Vet Res common., 27: 39-51.

Shotwell, O. L, Heseltine, C. W., Stubblefiel, R. D. and Sorenson, W. G. (1966): Production of aflatoxin on rice. Appl. Microbial., 14: 425-428.

Shouq K. M. (2004): Causes of vaccine failure in poultry. World veterinary Association.

Siegel, H. S., (1980): Physiological stress in birds. Bioscience, 30: 529-534.

Smith, H. W. and Hamilton, P. B. (1970): Aflatoxicosis in broiler chickens. Poultry Science, 44: 207-215.

Smith, J.W. and Hamilton, P.B (1970): Aflatoxicosis in the broiler chickens. Poultry science, 49: 207 – 215.

Snedecor, G. W. and W. G. Corchran (1980): Statistical Methods. Iowa State University Press, Ames, IA.

Soares, L. M. and Rodriguez – Amya, D. B. (1989): Survey of aflatoxins, ochratoxin A, Zeralenone and steringmatocystin in some brazilian foods by

using multi-toxin thin- layer chromatographic method. Journal of the Associatio official Analytical Chemists, 72: 22-26.

Stephen, J. F. and Barnett, B. D. (1964): Concurrent Salmonella typhimurium and Eimeria necatrix infections in chicks. Poultry Science, 45: 335-356.

Stroom, J. H. V. D. and Sluis, W.V.D. (1999): The effect of intercurrent disease on coccidiosis. Elcano Animal Health, Netherlands, 15-17.

Swayne, D. E., (2007): Understanding the complex pathogenicity avian influenza virus in birds. Avian disease, 5: 242 – 249.

Talkington, F. D. and Kelven, S. H. (1985): Evaluation of protection against colonization of the trachea following administration of Mycoplsama gallisepticum bacteria. Avian Disease, 29: 988-1003.

Talukder, M. H., Karim, M. J., Rahman, M. T. and Hossain, M. (2002): Effects of concurrent infections of Eimeria tenella with some bacteria and viruses in broiler chicks Bangladesh Vetenarian, 17: 21-26.

Tamara, I., Milijana, K., Sanja, A. K., Nesic, V. and Sanda, D. (2004): Immunohistochemical investigation of the bursa of fabricius in chicken experimentally infected by Eimeria Tenella. Acta Veterinaria, 54: 411-417.

Tessari, E.N., Oliveira, C.A., Cardoso, A.L., Ledoux, D.R. and rotlinghaus, G.E. (2006): Effects of aflatoxin B1 and fuminsin B1 on body weight, antibody titres and histology of broiler chicks. Britch Poultry Sci., 47: 357 – 364.

Thaxton, J. P., Tung, H.T. and Hamilton, P. B., (1974): Immunosupression in chickens by aflatoxin poultry Sci. 53: 721-725.

Thompson, E. D. and Lippman, M.E. (1974): Progress in endocrinology and metabolism: Mechanism of action of gluco corticoids. Metabolism 23: 159 – 202.

Tung, H. T., Smith, J. W. and Hamilton, P. B. (1971): Aflatoxicosis and braising in the chicken. Poultry sci 50: 795-800.

Van Der Goot J. A., Koch. G., De Jong. M.C. and Van Boven M. (2005): Quantification of the effect of vaccination on transmission of avian influenza (H7N7) in chickens. Proc. Natl. Acad. Sei. USA., 102: 18141-6.

Verma, J., Johri, T. S., Swain, B. K. and Amina, S. (2004): Effects of graded levels of aflatoxin, ochratoxin and their combination on their performance and immune response of broilers. Britch poultry science, 45: 512-518.

Viridi, J. S., Tiwari, R. P., Saxena, M., Khanna, V., Singh, G., Saini, S. S. and vadehra, D. V. (1989): Effect of aflatoxin on the immune system of the chick. J. Appl. Toxical., 9: 271 – 275.

Wanis E. I, Derhall, E. L., Nassar, A. M., Ebiary E. L., Asely, E., Hassan, N. and Barhouma, N. (1991b): Effect of infection with Eimeria necatrix on the response of chickens to NDV vaccine using lentogenic Hitcher (HBI) strain of NDV. Assiut Veterinary Medical Journal, 25: 80-88.

Wanis, E. I., Dehali, E. L., Nassar, A. M., Ebiary. E. L., Asely, E., Hassan, N. and Barhoum, N. (1991a): Effect of infection with Eimeria necatrix on the response of chickens to ND vaccine using mesogenic Komarove "K" strain of NDV. Assuit Veterinary Medical Journal, 26: 124-137.

West, S., Wyatt. R. D. and Hamilton, P. B. (1973): Increased yield of afaltoxin by incremental increases of temperature. Appl. Microbiol., 25: 1018-1019.

Williams, R. B. (2001): Quantification of the crowding effect during infections with the seven Eimeria species of the domesticated fowl: its importance for experimental designs and the production of oocysts stock. Journa of Parasitology, 31: 1065-1069.

Wyatt, R. D., Ruff, M. D. and page, R. K (1975): Interaction of aflatoxin with Eimeria tenella infection and Monesin in young broiler chickens. Avian, Dis., 19: 730-739.

تأثير كل من الأفلاتوكسين B1 الإيميريا تينيلا الميكوبلازما جليسيبتكم على الاستجابة المناعية ضد لقاح أنفلونزا الطيور الميت فى دجاج التسمين

خالد مدين دياب

قسم أمراض الدواجن – كلية الطب البيطرى – جامعة القاهره

الملخص العربى

أجريت هذه الدراسة لدراسة تأثير الأفلاتوكسين ، الإيميرياتينيلا, الميكوبلازما جليسيبتكم على الكفاءة الإنتاجية ورد الفعل المناعي لدجاج التسمين المحصن بلقاح الأنفلونزا الميت لإجراء الدراسة تم استخدام عدد 240 كتكوت تسمين عمر يوم وقد تم تقسيمهم عشوائيا إلى عدد ستة مجموعات متساوية : المجموعة (ا) استخدمت كمجموعة ضابطة سلبية (بدون أى معاملات ولم تحصن ضد أنفلونزا الطيور) المجموعة (ب) استخدمت كمجموعة ضابطة إيجابية (بدمن أى معاملات وحصنت بلقاح أنفلونزا الطيور الميت) ، المجموعة (ج) وتم تغذية الطيور بها على عليقه ملوثة صناعيا بسم الأفلاتوكسين بجرعة 2 مج / كجم عليقه من سن يوم حتى سن 42 يوم، المجموعة د تم إحداث العدوى التجريبية بها بالكوكسيديا الأعورية عند عمر 15 يوم عن طريق تجريع الطيور بالفم بحويصلات الإيميرياتينيلا بجرعة (20000 حويصلة/طائر)، المجموعة (ه) تم بها إحداث العدوى التجريبية بالكوكسيديا الأعورية بنفس جرعة حويصلات الإيميريا تينيلا المستخدمة فى المجموعة (د) ولكن عند عمر 21 يوم ، والمجموعة (و) وقد تم بها إحداث العدوى التجريبية بميكروب الميكوبلازما جليسيبتكم عن طريق الأنف بجرعة 8 X 10^4 لكل طائر عند اليوم الخامس عشر من العمر 0 تم تحصين جميع الكتاكيت فيما عدا الكتاكيت الموجودة بالمجموعة (ا) بلقاح أنفلونزا الطيور الميت عن طريق الحقن فى العضل عند اليوم السابع من العمر 0 تم تقييم رد الفعل المناعى تجاه لقاح الأنفلونزا الميت بقياس مستوى الأجسام المناعية فى المصل أسبوعيا ولمدة أربعة أسابيع بعد التحصين باستخدام كلا من اختبار مانع تلازن الدم واختبار الإليزا ، وقد تم أيضا تقييم الكفاءة الإنتاجية لطيور التسمين فى المجموعات التجريبية المختلفة بتسجيل معدل الوزان ومعدل اكتساب الوزن وكذلك معدل التحويل الغذائي عند نهاية التجربة (42 يوم)

أظهرت النتائج حدوث انخفاض معنوي فى مستوى الأجسام المناعية فى الدجاج فى المجموعات ج ، د ، ه ، ، و بالمقارنة بالمجموعة ب 0 وأظهر التحليل الإحصائي للنتائج أن تأثير الأفلاتوكسين على رد الفعل المناعى تجاه التحصين للقاح الأنفلونزا أكبر بصورة معنوية من كلا من تأثير الإيميرياتينيلا الميكوبلازما جليسيبتكم على الترتيب 0 أظهرت النتائج أيضا أن العدوى المبكرة بالإيميريا تينيلا أى عند عمر 15 يوم قد أثرت سلبيا على رد الفعل المناعي بصورة أكبر من تأثير إحداث العدوى بها عند عمر 21 يوم

وقد خلصت هذة الدراسة إلى أن وجود سم الأفلاتوكسين فى العلف وعدوى الإيميريا تينيلا و الميكوبلازما جليسيبتكم يؤدى إلى انخفاض رد الفعل المناعى تجاه لقاح أنفلونزا الطيور الميت فى دجاج التسمين وهو ما يشير إلى أن كلا منهم له تأثير مثبط لجهاز المناعة فى الدجاج وذلك التأثير قد يؤدى إلى حدوث وباء أنفلونزا الطيور فى القطعان المحصنة بطريقة صحيحة بلقاح أنفلونزا الطيور الميت