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The Effect of Mycotoxins on Immune Response of Broilers to the Live ND Vaccines Applied by Different Routes

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ABSTRACT

A study was conducted to assess the impact of mycotoxins naturally contaminated ration on the immune response of vaccinated broilers against Newcastle disease (ND)with live ND vaccines applied by different routes.150 one day old commercial chicks were divided into 5 equal groups (30 birds in each)according different routs of administration. Exposure of broilers to Ochratoxin with level of 23 part per billion (PPB) and Aflatoxin with level of 16 PPB resulted in significance decrease in immune response (humoral and cell mediated)with mean HI log_2antibody titers at the 66th day old (7.67) in both of vaccinated groups without mycotoxins consumption compared with (6.33) in spray vaccinated group with mycotoxin consumption and(5.67)in water vaccinated group with mycotoxin consumption. Also consumption of such contaminated ration resulted in lack of adequate protection against subsequent experimental challenge with VVND virus where the mortality was higher in groups fed in contaminated ration than in groups fed on mycotoxin free rations in both 1st and 2nd challenges with mortalities percentage 30% then 10%,60% then 50%, and 70% then 50% at both vaccinated groups without mycotoxins consumption, spray vaccinated group with mycotoxins consumption, and water vaccinated group with mycotoxin consumption in order. All results were in comparing with –Ve control group.

***Key words*:** Mycotoxins, Newcastle disease, LaSota, Immune response, vaccination.

Introduction

ND is a highly contagious and widespread disease of the avian species causing severe economic losses in poultry industry (Alexandar, 2001) Newcastle disease virus (NDV), also known as avian Paramyxovirustype-1 (AMPV-1), is a member of the Avulavirus genus within the Paramyxoviridae family (Alexandar,2003). Despite the all prevention and control measures including vaccination applied against ND, it still one of the most important disease in poultry production worldwide (Czegledi *et al.,* 2006). At the present time, vaccination programs for NDV include the use of lentogenic strains either inactivated (killed) or live. Among live vaccines marketed worldwide, the lentogenic Hitchner B1, LaSota, Clone 30 and VG/GA strains (genotype II) of NDV are currently the most widely efficaciously used for prevention of ND*(* Fabienne Rauw *et al.,*2009).Both cell-mediated and humoral immunity play a role in the protective immune response against NDV. Although high levels of systemic antibody have always been associated with protection against ND (Van Boven *et al.,* 2008;Kapczynski and King .,2005- Beard and Brugh, 1975) reports have suggested that antibody titer in serum measured by haemagglutination inhibition (HI) test are not directly correlated with the level of resistance of chickens to experimental NDV challenge (Gough and Alexander,1973; Reynolds and Maraqa,2000). The cellmediated immunity is not sufficient by itself to protect against virulent APMV-1,but may be essential for virus clearance (Russell *et al.,*1997).Immunosuppression in chickens can be caused by several factors such as natural, nutritional, manage mental, diseases, stress etc. mycotoxins have a significant effect on bird health due to their interference with vaccination programs (Azzam and Gabal, 1997; Gabal and Azzam, 1998).Consumption of mycotoxins, at levels that do not cause clinical mycotoxicosis, suppress immune functions and may decrease resistance to infectious disease. The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the vulnerability of the continually proliferating and differentiating cells that participate in immune mediated activities and regulate the complex communication network between cellular and humoral components.

Effect of mycotoxins on the avian immune system can be summarized in the following ; depressed T- or B- lymphocyte activity (regressed bursa and thymus), suppressed immunoglobulin and antibody production,

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reduced complement or interferon activity, impaired macrophage-effector cell function, and Reduced antibody titers and serum concentration of antibiotics (Ursula Hofstetter,2007).

Materials and Methods

Experimental design:

A (150) one day old broilers commercial chicks were initially divided into 3 main groups at 1st day ; (30) as a negative control (–Ve C) group fed on a normal commercial poultry ration treated with antimycotoxins feed additives throughout the experiment and without any vaccination , (60) as a mycotoxins (M) group fed on a mycotoxins contaminated commercial poultry ration with level of contamination of (23 PPP Ochratoxin and 16 PPP Aflatoxin) and with vaccination protocol as will mentioned bellow and (60) as a positive control (+Ve C) group fed on a normal commercial poultry ration treated with antimycotoxins feed additives throughout the experiment and with the same vaccination protocol applied at the M group.At the 7th day the M group were divided into two equal groups; (30) as a spray mycotoxin (S M) group in which ND vaccination protocol applied by spray rout of administration, and (30) as a water mycotoxin (W M) group in which ND vaccination protocol applied by water rout of administration. Also the (+Ve C) group were divided into two equal groups; (30) as a spray mycotoxin (SC) group in which ND vaccination protocol applied by spray rout of administration, and (30) as a water mycotoxin (WC) group in which ND vaccination protocol applied by water rout of administration. At the 30th day (and before application of the 2nd LaSota vaccination according to the protocol will mentioned bellow) ten (10) hens from each group were isolated to be challenged with the virulent ND virus at 37th day (21th day post 1st LaSota vaccination).At the 55th day (21th day post 2nd LaSota vaccination) ten (10) hens from each group were challenged with the virulent ND virus. Nearly in weekly basis whole blood samples were collected for HI test at 11 time points; 1st , 7th, 11th, 16th, 20th, 27th, 34th *, 42th, 49th, 56th, and 66th days.Also heparinized blood samples were collected for lymphocyte blastogenesis assay and phagocytic activity test at 8 time points ; 2nd, 14th, 22th, 29th, 36th, 43th, 50th, and 58th days. At the 70th day (the end of the 2nd challenge) the experiment were terminated and hens were slaughtered .

ND Vaccines:

Both Hitchner B1 (HB1) – batch No.24- and LaSota – batch No.22- live lyophilized vaccines vials are obtained from VSVRI and applied according to the instruction of the producer at 8th day for (HB1) and at 16th and 30th days for LaSota.

Infectious bursal disease (IBD) vaccines:

Both IBD (D78) strain – batch No.12-and IBD bursavacc strain – batch No.18-live lyophilized vaccines vials are obtained from VSVRI and applied as the instruction of the producer at 12th day for the bursavacc strain and at 22th day for theD78 strain.

Virulent NDV:

The virus used for challenge test throughout this experiment .It is VVNDV field isolate, was obtained from the VSVRI, Abbasia, Cairo. Its infectivity titer was 106 EID50/Dose (Reda and Sheble, 1976) used for evaluation of the potency of different type of NDV vaccines using challenge test according OIE-Manual, (2012).

Haemagglutinating antigen:

A commercial LaSota NDV vaccinal strain was propagated in allantoic sac of SPF-chicken embryos and used as antigen forHaemagglutination inhibition (HI) test which was carried out following the recommendation of OIE-Manual, (2012)on serum samples.

Experimental chicks:

(150) One-day-old commercial broilers chickens were purchased from (a local hatchery). They were floor reared, fed on commercial mycotoxin contaminated poultry ration, and kept under strict hygienic measures throughout the experiment.

Heparinized blood samples:

Jugular blood samples from chicks all groups were collected with anticoagulant (Heparin 20-40 IU/ml) for evaluation of cell mediated immune response by lymphocyte blastogenesis using XTT assay according to Mayer *et al.,* (1974), Lucy, (1977), Lee, (1984), and Scudiero *et al.,* (1988).And by Evaluation of phagocytic activity according to Antley and Hazen, (1988), and according Harmon and Glisson, (1989), which was modified by (Hussien, 1989), and according to Richardson and Smith, (1981).

Ration:

Mycotoxins contaminated ration with contamination levels; 23 PPPO chratoxin and 16 PPP Aflatoxin previously examined bylaboratory of mycotoxins diagnosis, faculty of veterinary medicine, Cairo university.

Statistical Analysis:

Data were presented as mean ± SD. One way analysis of variance (ANOVA) was chosen to compare mean differences between groups, Least Significant Difference (LSD) post hoc test was used for pair wise comparisons between groups when ANOVA is significant. The significance level was set as P value ≤ 0.05 considered significant. Statistical analysis was performed using SPSS version 16®.

Results

Humoral immune response:

Results of HI test for different groups presented in table (1), and chart (1):

At 20th, 49th, 56th, and 66th days of age there are a significant increase in both vaccinated fed on mycotoxins uncontaminated ration groups than both vaccinated fed on mycotoxin contaminated ration groups. At 34th day (4 days post 2nd LaSota vaccination), there are a significant increase in spray vaccinated fed on mycotoxins uncontaminated ration group than spray vaccinated fed on mycotoxin contaminated ration group with high significant increase at the 42th day. At 34th day there are a significant increase in spray vaccinated fed on mycotoxins uncontaminated ration group than water vaccinated fed on mycotoxins uncontaminated ration .

Table 1: HI log_2mean titers.

Effect of mycotoxins on HI log_2 mean titers in different groups.					
		M groups.		+Ve C groups.	
	-Ve C	WM	SM	WC	SC
1st day	4.67 ±0.82 a	4.97 ±0.76 a	4.97 ±0.76 a	4.75 ±0.87 a	4.75 ±0.87 a
7th day	3.80 ±0.84 b	4.87 ±0.74 a	4.60 ±0.91 ab	4.20 ±0.84 ab	4.40 ±0.55 ab
11th day	3.40 ±0.55 c	4.87 ±0.74 ab	4.40 ±0.63 b	5.40 ±0.55 a	5.40 ±0.89 a
16th day	2.00 ±1.00 d	4.93 ±0.80bc	4.47 ±0.64 c	5.60 ±1.14 ab	6.40 ±0.89 a
20th day	0.40 ±0.55 c	5.40 ±0.70 b	5.60 ±0.70 b	6.40 ±0.55 a	6.60 ±0.55 a
27th day	0.20 ±0.45 b	6.60 ±1.14 a	7.00 ±0.71 a	7.00 ±1.22 a	7.60 ±0.55 a
34th day	0.00 ±0.00 c	7.20 ±0.45 b	7.60 ±0.55 b	7.40 ±0.55 b	8.60 ±0.55 a
42th day	0.00 ±0.00 d	7.80 ±0.45bc	7.20 ±0.45 c	8.20 ±0.84 ab	9.00 ±1.22 a
49th day	0.00 ±0.00 c	6.60 ±0.55 b	6.80 ±0.84 b	8.40 ±0.89 a	8.60 ±0.55 a
56th day	0.00 ±0.00 c	6.33 ±0.58 b	6.67 ±0.58 b	8.33 ±0.58 a	8.67 ±0.58 a
66th day	0.00 ±0.00 c	5.67 ±0.58 b	6.33 ±0.58 b	7.67 ±0.58 a	7.67 ±0.58 a

Means with different letters (a, b, c, d) within the same row are significantly different at P value ≤ 0.05.,-Ve C : group without any vaccination and fed on mycotoxins uncontaminated ration, +Ve C : groups had vaccinated and feed on mycotoxins uncontaminated ration. W C : a +Ve C group, ND vaccinated through drinking water,S C : a +Ve C group, ND vaccinated through spraying, M : vaccinated groups fed on mycotoxins contaminated ration, W M : a M group, ND vaccinated through drinking water, S M : a M group, ND vaccinated through spraying.

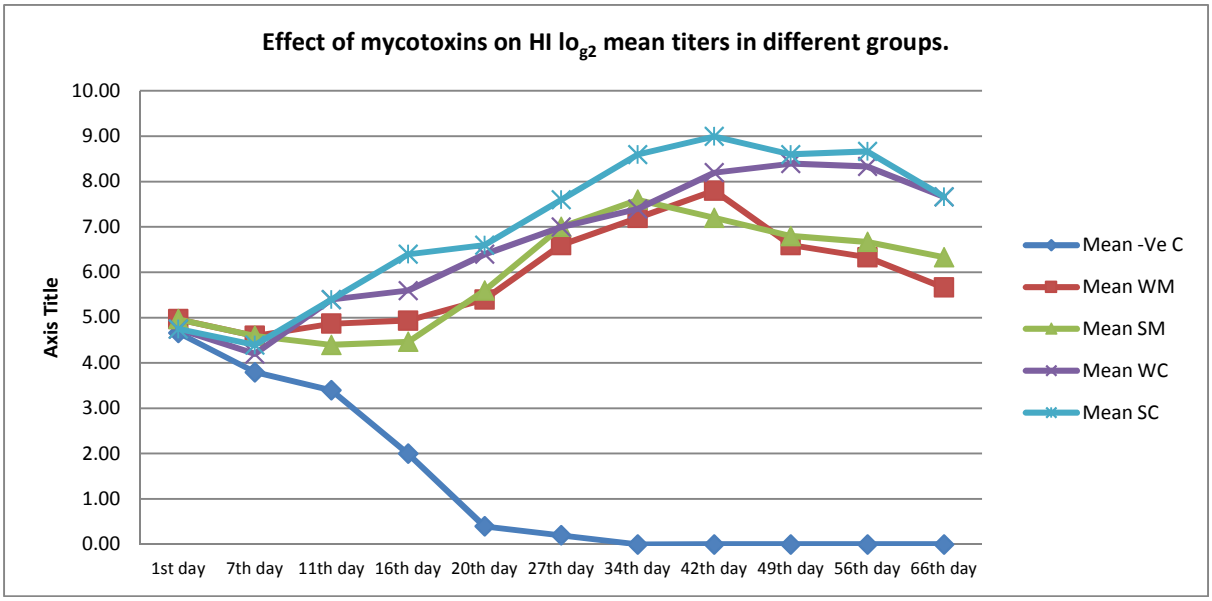

Chart 1: Humoral immune response.

Cell Mediated Immunity response:
I-Lymphocyte blastogenesis assay:
Results of Lymphocyte blastogenesis assay for different groups presented in table (2), and chart (2).

At 43thand at 50thday there are a significant increase in spray vaccinated fed on mycotoxins uncontaminated ration group than spray vaccinated fed on mycotoxin contaminated ration group. At 58th day there are a significant increase in water vaccinated fed on mycotoxin uncontaminated ration group than water vaccinated fed on mycotoxin contaminated ration group. At 50th day there are a significant increase in water vaccinated fed on mycotoxin contaminated ration group than spray vaccinated fed on mycotoxin contaminated ration group.

Table 2: lymphocyte blast genesis assay.

Effect of mycotoxins on Lymphocyte blastogenesis in different groups.					
	-Ve C	M groups.		+Ve C groups.	
		WM	SM	WC	SC
2nd day	0.75 ±0.04 b	1.06 ±0.10 a	1.06 ±0.10 a	0.67 ±0.09 b	0.67 ±0.09 b
14th day	0.43 ±0.03 b	1.28 ±0.09 a	1.49 ±0.08 a	1.36 ±0.11 a	1.51 ±0.32 a
22th day	0.50 ±0.07 b	1.08 ±0.08 a	0.95 ±0.07 a	1.04 ±0.09 a	1.12 ±0.14 a
29th day	0.31 ±0.05 a	0.50 ±0.10 a	0.45 ±0.03 a	0.79 ±0.51 a	0.68 ±0.05 a
36th day	0.21 ±0.01 c	0.73 ±0.15 b	0.82 ±0.07 b	1.24 ±0.18 a	1.25 ±0.09 a
43th day	0.19 ±0.03 c	0.54 ±0.09 ab	0.36 ±0.02 bc	0.69 ±0.08 a	0.73 ±0.13 a
50th day	0.16 ±0.03 a	0.12 ±0.02 ab	0.08 ±0.00 c	0.11 ±0.01 bc	0.12 ±0.01 ab
58th day	0.10 ±0.00 c	0.28 ±0.04 b	0.37 ±0.03 ab	0.40 ±0.04 a	0.47 ±0.08 a

Means with different letters (a, b, c) within the same row are significantly different at P value ≤ 0.05.

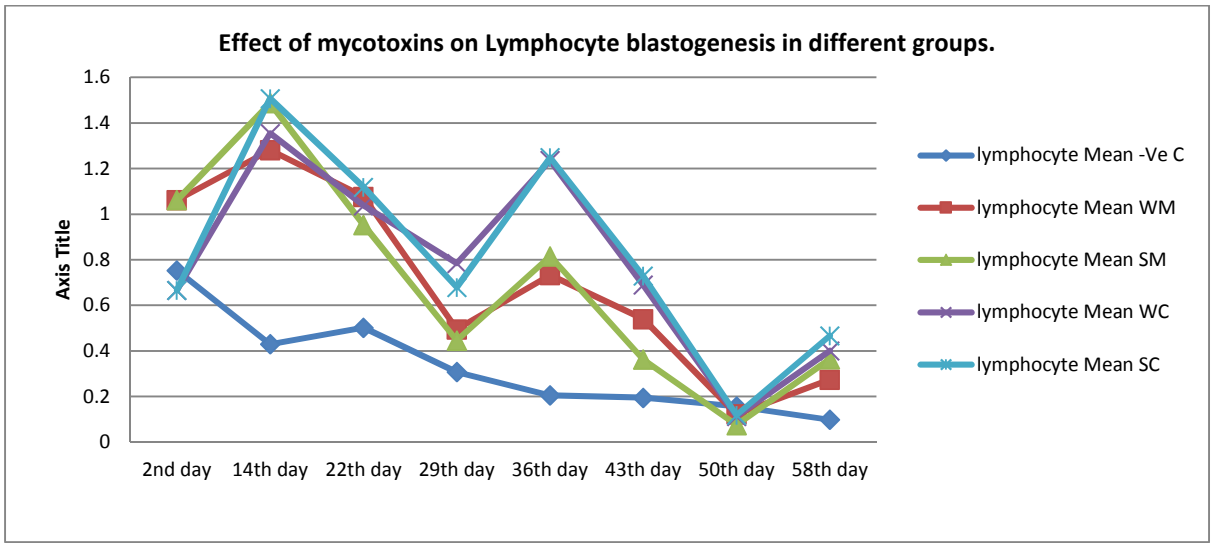

Chart 2: lymphocyte blastogenesis assay.

II-phagocytic activity:
a-phagocytic %:
Results of phagocytic %for different groups presented in table (3), and chart (3):

At 14thand 29thdaysthere are a significant increase in spray vaccinated fed on mycotoxins uncontaminated ration group than sprayvaccinated fed on mycotoxin contaminated ration group with high significant increase at 50th day. At 36thand 43thdaysthere is a significant increase in both vaccinated fed on mycotoxins uncontaminated ration groups than both vaccinated fed on Mycotoxin contaminated ration groups. At 50th day there are a significant increase in water vaccinated fed on mycotoxins contaminated ration than spray vaccinated fed on mycotoxins contaminated ration and with significant increase in spray vaccinated fed on mycotoxins uncontaminated ration group than water vaccinated fed on mycotoxins uncontaminated ration group.

b-phagocytic index:
Results of phagocytic index for different groups presented in table (4), and chart (4):

At 14thand 29thday there are a significant increase in spray vaccinated fed on mycotoxins uncontaminated ration group than spray vaccinated fed on mycotoxin contaminated ration group with high significant increase at the 50th day. At 36th day there are a significant increase in water vaccinated fed on mycotoxins uncontaminated ration group than water vaccinated fed on mycotoxins contaminated ration group. At 22th day

there are a significant increase in water vaccinated fed on mycotoxins uncontaminated ration group than spray vaccinated fed on mycotoxins uncontaminated ration group.

Table 3: Phagocytic %:

Effect of mycotoxins on phagocytic %in different groups.					
	-Ve C	M groups.		+Ve C groups.	
		WM	SM	WC	SC
2nd day	55.47% ±5.12 a	80.11% ±10.45 a	80.11% ±10.45 a	61.18% ±19.54 a	61.18% ±19.54 a
14th day	46.43% ±5.05 b	62.35% ±3.33 ab	46.67% ±18.86 b	59.76% ±.38 ab	81.68% ±3.10 a
22th day	53.08% ±9.79 b	61.28% ±11.24 ab	70.83% ±5.89 ab	74.09% ±6.87 a	70.09% ±1.89 ab
29th day	62.59% ±1.48 ab	52.27% ±3.21 ab	48.75% ±15.91 b	57.14% ±10.10 ab	72.50% ±3.54 a
36th day	51.67% ±2.36 b	52.79% ±0.22 b	57.64% ±4.91 b	64.85% ±0.21 a	68.99% ±0.81 a
43th day	30.36% ±7.58 c	30.54% ±0.33 c	39.90% ±1.43 bc	50.48% ±8.54 ab	59.55% ±0.64 a
50th day	23.63% ±4.30 c	37.74% ±8.12 ab	21.36% ±1.93 c	27.64% ±0.51 bc	41.04% ±0.19 a
58th day	22.65% ±0.60 a	21.14% ±3.38 a	18.41% ±4.27 a	25.10% ±2.22 a	23.61% ±1.96 a

Means with different letters (a, b, c) within the same row are significantly different at P value ≤ 0.05.

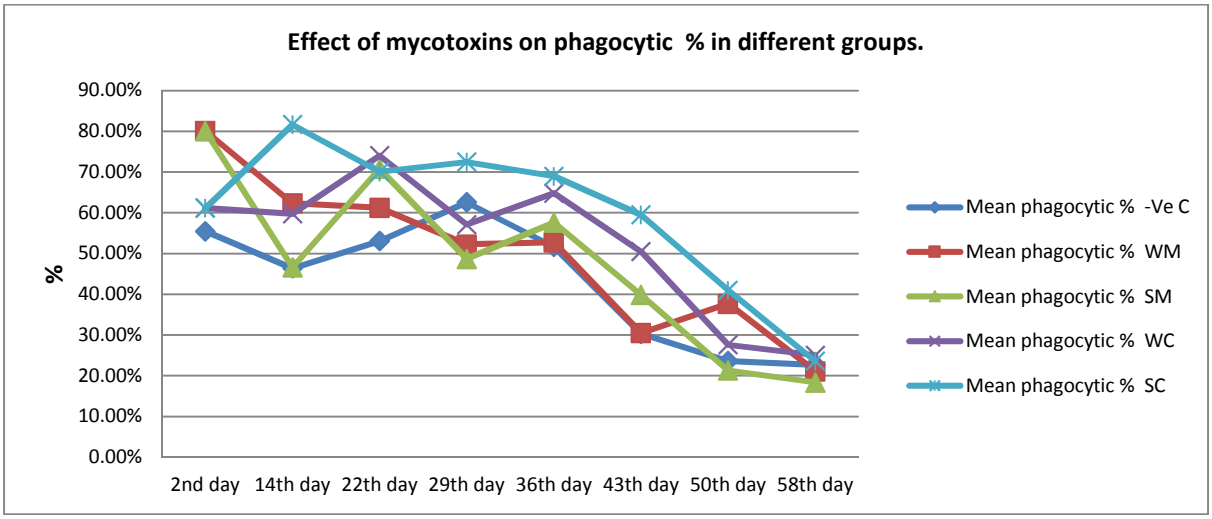

Chart 3: phagocytic % .

Table 4: phagocytic index.

Effect of mycotoxins on phagocytic index in different groups.					
	-Ve C	M groups.		+Ve C groups.	
		WM	SM	WC	SC
2nd day	0.45 ±0.01 a	0.56 ±0.09 a	0.56 ±0.09 a	0.55 ±0.11 a	0.55 ±0.11 a
14th day	0.33 ±0.06 c	0.41 ±0.01bc	0.37 ±0.05 c	0.51 ±0.01 ab	0.60 ±0.06 a
22th day	0.26 ±0.10 c	0.54 ±0.00 ab	0.61 ±0.07 ab	0.69 ±0.01 a	0.52 ±0.06 b
29th day	0.47 ±0.11 a	0.43 ±0.10 ab	0.28 ±0.04 b	0.54 ±0.05 a	0.58 ±0.03 a
36th day	0.20 ±0.00 c	0.27 ±0.14bc	0.40 ±0.02 ab	0.49 ±0.02 a	0.52 ±0.00 a
43th day	0.13 ±0.01 b	0.22 ±0.01 ab	0.30 ±0.03 ab	0.39 ±0.24 ab	0.49 ±0.06 a
50th day	0.08 ±0.03 c	0.15 ±0.04bc	0.12 ±0.04bc	0.19 ±0.01 b	0.34 ±0.02 a
58th day	0.17 ±0.08 a	0.12 ±0.08 a	0.18 ±0.04 a	0.25 ±0.02 a	0.21 ±0.06 a

Means with different letters (a, b, c) within the same row are significantly different at P value ≤ 0.05.

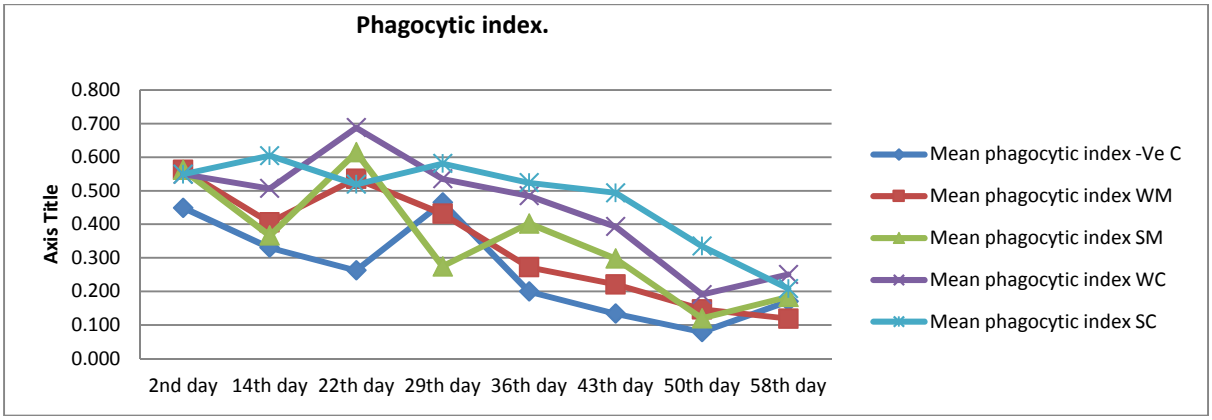

Chart 4: phagocytic index.

Protection against VVND:

Challenge test results presented at table (5), and chart(5):

Protection rate was ;0%, 30%, 40%, 70%, and 70% in groups –Ve C, W M, S M, W C, and S C respectively at 1st challenge and 0%, 50%, 50%, 90%, and 90% in the same order at 2nd challenge .

Table 5: challenge results.

Effect of mycotoxins on protection rates against VVND in different groups.										
	-Ve C		M groups.				+Ve C groups.			
			WM		SM		WC		SC	
Challenge No.	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
% dead	100%	100%	70%	50%	60%	50%	30%	10%	30%	10%
% protection	0%	0%	30%	50%	40%	50%	70%	90%	70%	90%

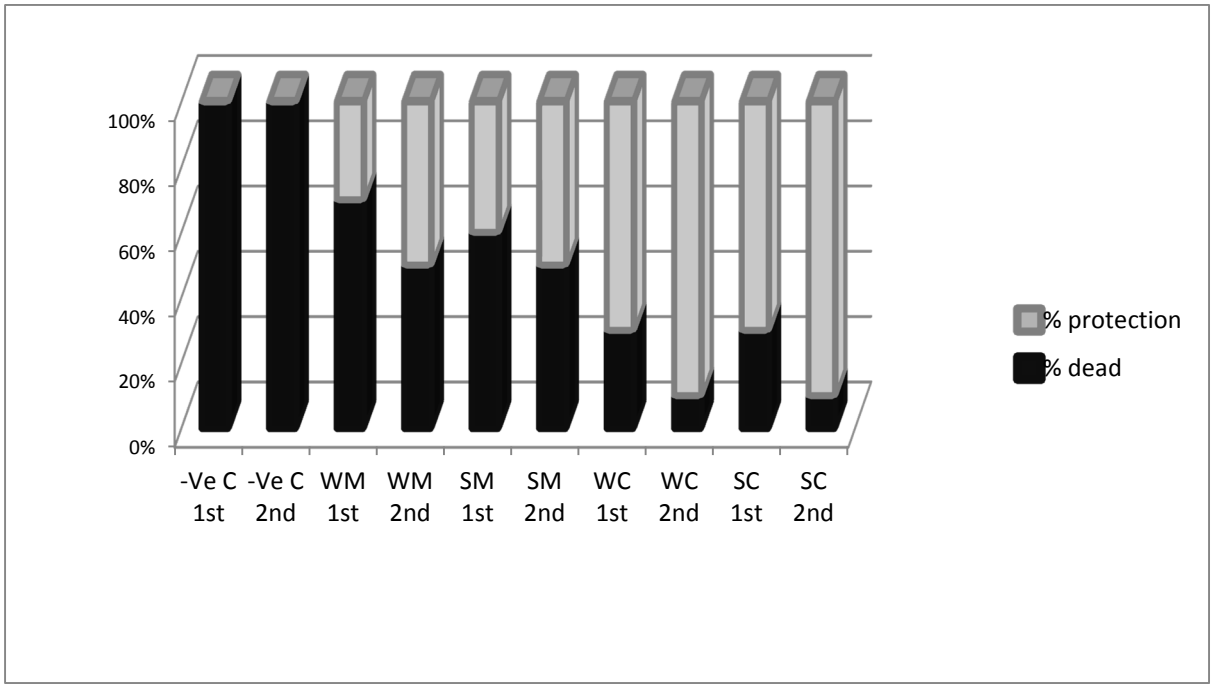

Chart 5: Protection rates.

Discussion

In this study, the effect of naturally mycotoxin contaminated ration on the immune response of commercial broilers to LaSota ND vaccine was studied by evaluation of humoral and cell mediated immunity .In addition, the effect of mycotoxin contaminated ration on the LaSota ND vaccine protection rate was estimated.

H I testwas conducted to evaluate the humoral immune response, both lymphocyte blastogenesis assay and phagocytic activity test to evaluate the (CMI) response, and challenge test to evaluate the potency of the used LaSota ND vaccine.

Concerning mycotoxin effect on H I results we found that both vaccinated (Water and Spray) fed on mycotoxins uncontaminated ration groups have a significant increase in mean AB titers at 20th day (6.4 and 6.6), 49th day (8.4 and 8.6), 56th day (8.33 and 8.67), and 66th day (7.67 and 7.67) comparing with both vaccinated (Water and Spray) fed on mycotoxins contaminated ration groups (5.4 and 5.6), (6.6 and 6.8), (6.33 and 6.67) and (5.67 and 6.33) which is supported by with (Campbell *et al.,* 1988; Singh *et al.,* 1990; Gabal and Azzam, 1998; Elizabeth Santin *et al.,* 2002 ; Elaroussi *et al.,* 2006 ; Ebrahimi and Shahsavandi, 2008).

AB titers noticed to be decreased in mycotoxins contaminated rations groups which may be due to the fact that mycotoxins delays or decreases antibody titres, specifically of agglutinins or haemagglutinins, and also levels of certain serumimmunoglobulins e.g. IgG and IgA(Thurston *et al.,* 1974; Campbell *et al.,* 1988)and other mechanisms (Pier *et al.,* 1979).

Cell-mediated immune response may be detected as early as two to three days after vaccination with alive vaccine (Reynolds and Maraqa, 2000).

Concerning mycotoxin effect on results of CMI estimation tests we found that at 14th and 29thdays spray vaccinated fed on mycotoxins uncontaminated ration group has a significant increase in phagocytic activity - % and index -which revealed (81.68% and 0.6) and (72.5% and 0.58) than spray vaccinated fed on mycotoxins contaminated ration group which revealed (46.67% and 0.37) and (48.75% and 0.28). At 36th and 43th days both vaccinated (Water and Spray) fed on mycotoxins uncontaminated ration groups have a significant increase in phagocytic activity - % only – which revealed (64.85% and 68.99%) and (50.48% and 59.55) comparing with both vaccinated (Water and Spray) fed on mycotoxins contaminated ration which revealed (52.79% and 57.64 %) and (30.54% and 39.9%) .At 50th day spray vaccinated fed on mycotoxins uncontaminated ration group has a significant increase in lymphocytes which revealed (0.12) and a high significant increase in phagocytic activity (% and index) which revealed (41.04% and 0.34) than spray vaccinated fed on mycotoxins contaminated ration group which revealed (0.08) and (21.36% and 0.12). That all support the fact that mycotoxins affect some immunity mechanisms by inhibition of macrophage migration by reduction in macrophage motility , affect mitogen responsiveness Pier *et al.,*(1979), interfere with the haemolytic activity of complement, reduction in the number of lymphocytes through its toxic effect on the Bursa of Fabricius, and impairment of cytokines formation by lymphocytes (Thurston *et al.,* 1974; Campbell *et al.,* 1988; Singh *et al.,* 1990 ; Azzam and Gabal, 1997;Ursula Hofstetter.,2007) and causing dramatic hypoplasia of the thymic cortex, a quantitative suppression of lymphokine production by T cells , inhibit phagocytosis with impaired phagocytosistosis by heterophils so subsequent diminution of antigen presentation by affected macrophages to the lymphocyte pool, depress blood monocyte phagocytic activity(Hofstetter, 2007).

Concerning protection rates vaccinated (Water and Spray) fed on mycotoxins contaminated ration groups have a lower protection rates which were (30% an 40%) and (50% and 50%) at both 1st and 2nd challenges than vaccinated (Water and Spray) fed on mycotoxins uncontaminated ration groups which revealed (70% and 70%) and (90% and 90%) which supports the suggestions of Ragland *et al.,*(1998); Elizabeth Santin *et al.,*(2002)that mycotoxins of various types reduce the immune response and thus are likely to increase the susceptibility of birds to infections and decrease the response to vaccines.

Conclusion

Therefore mycotoxins are a potent immune suppressive interfere with broilers immune response to live ND vaccines.

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