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Original Research Article

Studies on the effect of different immunostimulants on chick's immune response to inactivated avian influenza and Newcastle Vaccines

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ABSTRACT

Newcastle Disease virus (NDV) and Avian Influenza virus (AI) are represent a great negative significant causing severe economic losses and increased mortalities worldwide. Newcastle disease (ND) and Avian Influenza (AI) vaccination were targeting to lower the losses from mortality, reduce the viral load in the environment as well as eradication of positive cases. Many immunostimulants had been used to improve the immune response of vaccinated chickens. The current study was designed to compare the effect of different immunostimulants on chick's immune response to bivalent ND with AI-H5N1 oil vaccine. One hundred and ten, 1- day old Baladi chicks, At the 1st day of life (0 day) 10 birds were sacrificed to obtained individual blood samples for serum to determine maternal antibodies (MDAbs) to both AI and ND. Rest of birds (100 chicks) were divided into 5 equal groups (1-5); each 20 chicks. All chicken groups were vaccinated against ND with eye drop instillation of HB1 vaccine. While, at the 9th day birds of the groups 1-3 and 5 were given H5N1 vaccine by S.C injection, birds of group 4 were lifted as non AI vaccinated control. The used immune stimulants under test were given to groups 1, 2, and 3 as follows Lector, Superimmune and Imuvral; respectively. All the groups were subjected to daily observation with recording of feed intake, weekly body weight gain and total FCR, Weekly serum samples were collected, for serological examination, and the results showed high antibody titers, low mortality rates and better body performance in the groups treated with immunostimulants than the other groups which were not treated with the immunostimulants

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1. Introduction

NDV represents a negative input in the process of converting resources or production factors into products, goods and services available to people; it causes direct economic losses for the producer and a potential loss of value in the view of the consumer (Otte and Chilonda 2000). The aim of vaccine is to enhance immunity against infection and replication of the virus (Alexander, 1997), and to protect birds from the more serious consequences of NDV infection and clinical signs. However virulent ND strains may still infect, replicate, and be excreted from vaccinated birds (Capua et al., 1993).

Large numbers of poultry have died from HPAI or been culled to control the disease since it spread widely from 2004 onwards (Otte et al., 2008).

The effect of a mixture of some immunostimulant substances on the immune response of broiler chicks to bivalent AI-ND vaccine have a great significant as the injected immunostimulant mixture with the bivalent AI-ND vaccine give 100% protection against the challenge NDV, Whereas, 80 and 60% protection were obtained in chicks vaccinated either with AI-ND vaccine or live ND vaccines; respectively (Hussein et al., 2009). Antioxidant treatments improved the immune status as indicated by significantly higher HI titre of ND virus or the lymphoid organ relative weights such as thymus, bursa of Fabricius and spleen (Tollba et al 2007). The used immune stimulants composed of lectine, mixture of vitamins, Beta glucan and other constituents had different mechanisms in their action as immune stimulants due to its difference in their composition. Our study aimed to improve chick's immune response against ND&AI vaccination and improve its performance.

2. Material and methods

2.1. Chickens

One hundred and ten, 1- day old Baladi chicks ware obtained from commercial hatchery. The used chicks will be floored reared and fed on a balanced commercial ration. At the 1st day of life (0 day) 10 birds were sacrificed to obtained individual blood samples for serum to determine MDAbs to both AI and ND.

2.2. Ration

The chicks were feed on prepared ration according to the Ross broiler management manual and *NRC (1994)* requirement for broiler. All housed chickens were given ration adlibitum.

2.3. Vaccine Strains

Newcastle disease (ND) vaccine strains Hitchiner B1 Batch NO (19) exp. Date 12/2013, each vial contains virus titre of 10^9 EID_{50} was used after titeration for vaccination of experimental chicks via eye instillation route produced by vet. Ser. & vacc. Res. Inst.- Cairo – Egypt.

2.4. Avian Influenza vaccine

Oil adjuvant vaccine subtype H5N1 (Lot No: 2007013) produced by Harbin weike Biotechnology Development Co.China. It was used by subcutaneous route.

2.5. Bivalent AI and ND inactivated vaccine

The vaccine produced by Veterinary Research and Vaccine Cairo at 11/2009 and used by subcutaneous injection at the upper 3rd of the neck / bird 0.5 ml.

2.6. Velogenic NDVs

A local velogenic viscerotropic Newcastle disease virus (VVNDV) isolate *(Shible and Reda, 1976)* was kindly supplied by Newcastle Diseases Department; Veterinary Serum & Vaccine Research Institute, Abbasia, Cairo, Egypt to be used for challenge test.

2.7. Experimental design

One hundred chicks were divided into 5 equal groups (1-5); each 20 chicks. Each group was kept on floor in a separate clean disinfected pen and commercial ration and water adlebtum. All chicken groups were vaccinated against ND with eye drop instillation of HB1 vaccine. While, at the 9th day birds of the groups 1-3 and 5 were given H5N1 vaccine by S.C injection, birds of group 4 were lifted as non AI vaccinated control. The used immune stimulants under test were given to groups 1, 2, and 3 as follows Lector, Superimmune and Imuvral; respectively. All the groups were subjected to daily observation with recording of feed intake, weekly body weight gain to calculate weekly and total FCR, Weekly individual clotted blood samples for serum were taken, for serological testing using HI at 0,7, 16, 23 and 29 days of age (0, 1, 2 and 3 week post

vaccination). At 2, 3 and 4 of age 3 birds were killed with collection of blood for clinical pathological examination and also spleen, liver, kidney and thymus tissues in formal saline for histopathology.

2.8. Broiler performance parameters

In this study broiler growing performance parameters including average weekly mortality rate, body weight gain /gm., Feed intake/gm (CFI/gm), feed conversion rate (FCR) were used and calculated according to *Sainsbury, (1984).*

2.9. Red blood cells (RBCs)

Red blood cells from mature male chicken for Hemagglutination (HI) and Hemagglutination inhibition (HI) testing was performed with 0.5% chicken RBCs by a standard method according to the World Health Organization manual on Animal Influenza Diagnosis and Surveillance *(WHO, 2002).*

2.10. Hemagglutinating (HA) antigens

NDV HA antigens were prepared from La Sota vaccinal strain of NDV vaccine by passages in allantoic sac of 9-10 days-old embryonated chicken eggs. The amnio-allantoic fluids of infected embryos were collected aseptically, tested for HA and used as antigen in HI test *(Amer, 1984).* AI antigen was obtained from vaccine producer as inactivated lyophilized vials and titrated for HA unites using HA test.

2.11. Hematological Studies

Total erythrocyte and leukocyte counts were done using an improved Neubauer hemocytometer. Packed cell volume (PCV %) was estimated by microhematocrit technique. Hemoglobin concentration was colorimetrically determined using cyanmethemoglobin method. Differential leukocytic count was performed on Giemsa stained blood smears *(Feldman, et al., 2000).*

2.12. Immunostimulants

a) LECTOR 50® is a commercial feed additive product composed of lectine 50000 mg, Xylitol 20000 mg and Fructoligosaccharide 50000 mg Nacl 30000 mg and Dist. Water, obtained from microbiotech. USA (patch NO 3355). It was used in water at rate of 1 ml/ L.

b) SUPERIMMUNE® is a commercial water grade product from an immunostimulant whose formulation (vitamin A 10000000 IU, E 5000mg and C 10000 mg). REG No 5533 supplied by Pharmaceutical Industries Company for AM trade and was used in drinking water at rate of 1 ml/ L.

c) IMUVRAL® is composed Beta glucan 1700mg, mannanoilgosaccharide 300 mg problinglucol produced by CHEMVET used in drinking water at rate of 1 ml / L.

2.13. Histopathological Examination

Tissue specimens from bursa, thymus, and spleen of experimentally infected and control chicks were fixed in 10% formol saline for 24 hours. *Sections* of an average thickness of 3 microns was prepared and stained with hematoxyline and eosin according to *Banchroft et al. (1996).*

2.14. Statistical Analysis

Values were expressed as mean ± SD. Statistical comparisons among the means of different experimental groups were made with completely randomized two ways ANOVA "Student-Newman-Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance.

3. Results

The average weight gain (BWG), feed intake and feed conversion rate (FCR) of commercial chicks vaccinated with bivalent (AI+ND) are shown in Table (1). While table (2) showed that the ND-HI antibody titres of immunostimulant treated groups were higher than control at 2 and 4th week.

Regarding challenge test using vvND showed that (table 3) control –ve group showed 75% morbidity and 62.5% mortality i.e. the highest values those indicate the pathogenicity of use challenge virus as well as the more susceptibility of this group as compared with all vaccinated ones. Imuvral, Lector and vaccinated groups are showed morbidity rate 25% while diseased birds were detected earlier than treated control. No mortality was detected in Imuvral and Lector groups while Superimmune, control vaccine showed only one died bird followed by 5 in group 4.

Table 1. Average weight gain (BWG), feed intake and feed conversion rate (FCR) of commercial chicks vaccinated with bivalent (AI+ND).

GR. NO	VACCINE	IMMUNE STIMULANT	AGE / WEEK	BWG Mean ± SD		FI/ GM	FCR
1	(AI &ND)	Lector	1	15.32	±3.87	55.21	3.60
			2	33.65	±10.09	116.77	3.47
			3	46.79	±19.71	150.53	3.22
2	(AI &ND)	Superimmune	1	15.32	±3.87	55.21	3.60
			2	43.94	±12.21	108.35	3.19
			3	46.60	±21.43	138.12	2.96
3	(AI &ND)	Imuvral	1	15.32	±3.87	55.21	3.60
			2	36.78	±11.13	135.10	5.04
			3	48.76	±15.19	150.24	3.08
4	Control (Negative)		1	15.32	±3.87	55.21	3.60
			2	39.90	±13.87	109.32	2.74
			3	50.79	±18.31	140.54	2.77
5	Control (AI+ND)		1	15.32	±3.87	55.21	3.60
			2	37.21	±17.01	112.20	2.37
			3	47.38	±11.35	147.35	3.96

Table 2. HI antibody titres against AI and ND in vaccinated chicken groups (n=6).

Gr. NO	Vaccine	Immune stimulant	Age / Wks	AI- HI test									ND-HI test							
				Distribution of titre log 2 TRN							Mean ± SD		Distribution of titre log 2 TRN							Mean ± SD
				0-2	3	4	5	6	7	8			0	1	2	3	4	5	6	
1	(AI&ND)	Lector	0			1	2	3			5.3	±0.75					3		3	5.0 ±1.10
			1		1	3		2			4.5	±1.12			2	1	3			3.2 ±0.98
			2			1	1		1	3	6.7	±1.60				1		3	2	5.0 ±1.10
			3	1	1	1	1	2			4.0	±2.08	2	2	2					1.0 ±0.89
			4	3	2	1					2.7	±0.75		1	2	1		2		3.0 ±1.67
2	(AI&ND)	Superimmue	0			1	2	3			5.3	±0.75					3		3	5.0 ±1.10
			1		1	3		2			4.5	±1.12			2	1	3			3.2 ±0.98
			2		2	1	1			2	5.2	±2.11				2	1	3		4.2 ±0.98
			3	2	3	1					2.8	±0.69	2		3	1				1.5 ±1.22
			4	1	1		2				3.3	±2.05	1			1		1	1	3.5 ±2.65
3	(AI&ND)	Imuvral	0			1	2	3			5.3	±0.75					3		3	5.0 ±1.10
			1		1	3		2			4.5	±1.12			2	1	3			3.2 ±0.98
			2		2	3	1				3.8	±0.69				3	3			3.5 ±0.55
			3			1	3	2			5.2	±0.69	2	1	2		1			1.5 ±1.52
			4		2	2	2				4.0	±0.82		1	1		1	2	1	3.8 ±1.94
4	Control (Negative)		0			1	2	3			5.3	±0.75					3		3	5.0 ±1.10
			1		1	3		2			4.5	±1.12			2	1	3			3.2 ±0.98
			2		1	2	1	1		1	5.0	±1.63				2	3	1		3.8 ±0.75

		3			1	3	1			4.0	±0.63			1	2	2			1.5	±0.84
		4		2	3	1				2.8	±0.69		1	3	1				1.0	±0.71
5	Control (AI+ND)	0				1	2	3		5.3	±0.75						3		3	5.0	±1.10
		1			1	3		2		4.5	±1.12				2	1	3			3.2	±0.98
		2		1	1	3				3.4	±0.80				1	2	2	1		3.5	±1.05
		3		2	3	1				2.5	±1.38			5	1					1.2	±0.41
		4		2	1	3				2.5	±1.80		1	2	2	1				1.5	±1.05

Table 3. Daily distribution of morbidity and mortality in challenged chickens

Gr. NO	Vaccine	Immune Stimulant	Case No	Days post-challenge						Total NO	Percentage	Protection%
				3	4	5	6	7	8-21			
1	(AI+ND)	Lector	Dis.				1	1	-	2	25	
			Died	-	-	-	-	-	-	0	100	100 %
2	(AI+ND)	Superimmun	Dis.		1	2	-	-	-	3	37.5	
			Died				1	-	-	1	12.5	87.5 %
3	(AI+ND)	Imuvral	Dis.			1	1	-	-	2	25	100 %
			Died	-	-	-	-	-	-	0	100	
4	Control (Negative)		Dis.	1	3	2	1		-	6	75	
			Died		1	2	1	1	-	5	62.5	37.5 %
5	Control (AI+ND)		Dis.		1	1	-	-	-	2	25	
			Died			1	-	-	-	1	12.5	87.5 %

Calculated results of WBCs at 4 and 5 weeks post vaccination(wpv), of control vaccine (group 5) are significant higher than all immune treated and control –ve groups. Control –ve group also is higher than Imuvral, superimmune and Lector groups. RBCs of group 5 are higher than control –ve and superimmune. In the other hand lector group showed lowest significant than superimmune. Hb results of group 5 are higher than all groups (1:4). Values of lector are lower than superimmune and control groups (4 and 5). PCVs are passing the same way with RBCs count. Differential leucocytes showed higher values of eosinophiles in stimulants given groups than controls. Monocytes of Lector group at 3 week are higher than all groups (2: 5). In the 4th and 5th week control –ve group showed the highest significant values between all groups (table 5 fig. 6). Lymphocytes of Imuvral are significant higher at all intervals than control groups (4 and 5) while Lector treated group 1 showed also higher values than superimmune and Imuvral. Heterophiles of control –ve are higher than all throughout all intervals. Lector groups at 3, 4 and 5 weeks showed lower values than superimmune and Imuvral groups.

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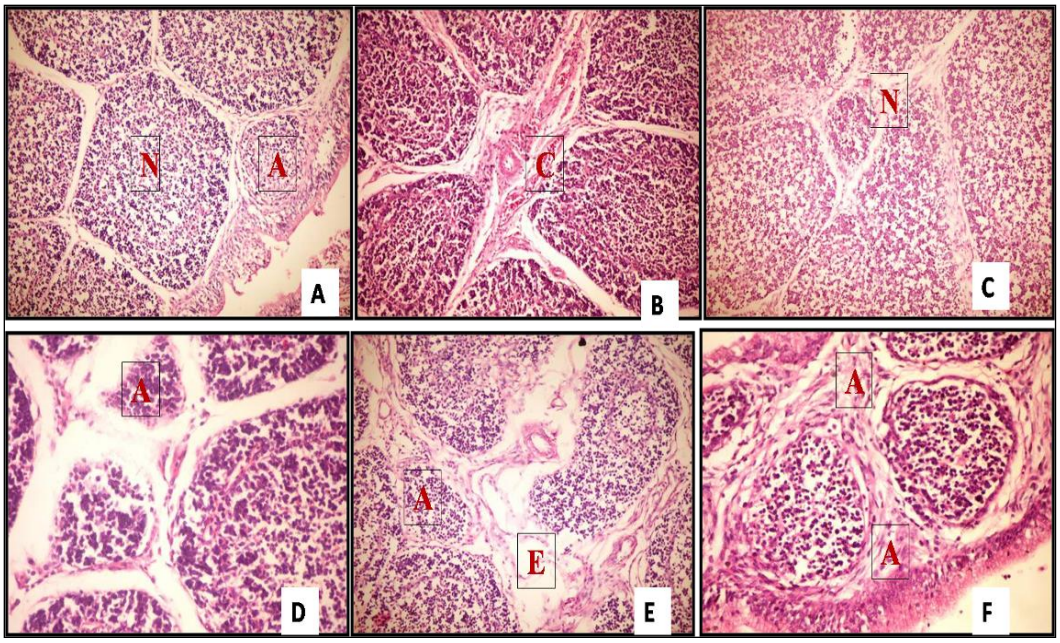

Figure 1. Bursal sections of chicken groups given immunostimulants with vaccines (H & Ex200) showing: A) Super (2wpv) necrosis of some follicles (N) and atrophy of others (A). B) AI +ND (2wpv) perifollicular congestion (C). C) Lector and super (3wpi) severely necrosed follicles (N). D) Imuvral (3wpv) follicular atrophy (A). E) AI + ND (4wpv) perifollicular edema (E) with follicular atrophy (A). F) AI + ND (5wpv) marked follicular atrophy (A).

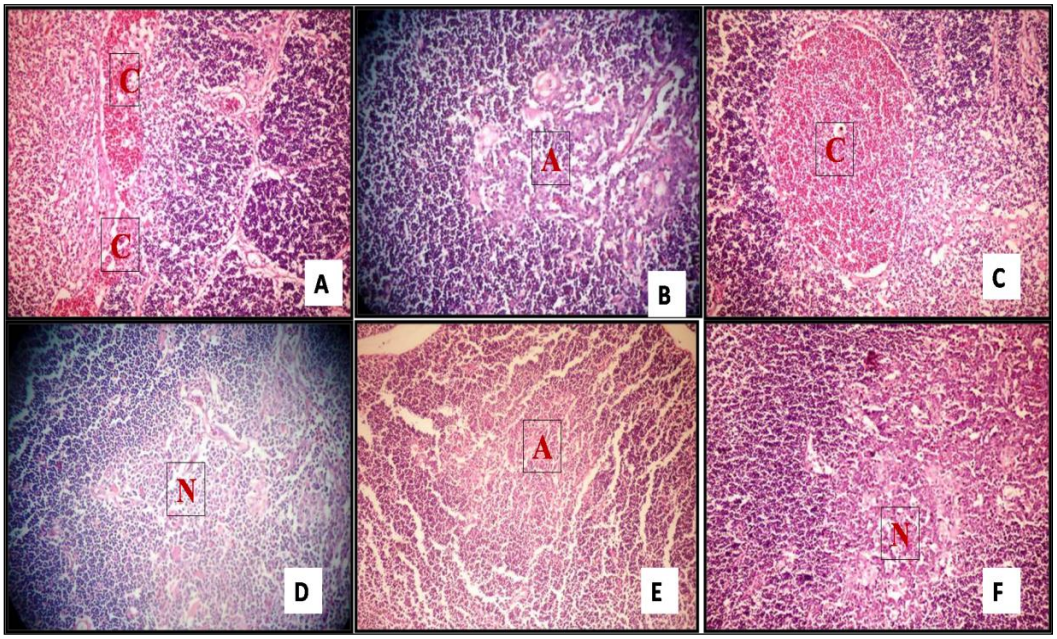

Figure 2. Thymus sections of chicken groups given immunostimulants with vaccines (H & Ex200). A) Lector (2wpv) medullary congestion (C). B) Super (2wpv) atrophied medulla (A). C) Lector (3wpv) severely congested medulla (C). D) Super (3wpv) necrosed medulla (N). E) Imuvral (3wpv) atrophied medulla, (A). F) AI + ND (5wpv) necrosed medulla (N).

Table (4): Hematologic picture in immunostimulant treated and vaccinated chicken groups.

Gr. NO	Vaccine	Immune stimulant	WBCs				RBCs				Hb				PCV			
			2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
1	ND+AI	Lector	55.0±3.61	70.0±10.97	80.3±0.88$^{\#bc}$	72.0±1.15$^{\#c}$	3.03±0.22	3.52±0.15	2.69±0.13$^{\#c}$	2.69±0.13$^{\#c}$	9.7±0.71	8.7±0.29$^{\#ab}$	8.7±0.15$^{\#c}$	7.7±0.29$^{\#c}$	29.3±2.19	26.0±1.15$^{\#ab}$	26.0±0.58$^{\#c}$	23.7±0.88$^{\#c}$
2	ND+AI	Superimmune	55.33±6.77	63.0±6.93	78.0±0.58^{bc}	73.0±2.52^{c}	2.70±0.12	2.99±0.03	3.34±0.12^{ab}	3.34±0.12^{ab}	8.5±1.03	8.0±0.17^{b}	10.4±0.2^{a}	9.0±0.09^{ab}	25.7±2.96	24.0±0.58^{b}	30.3±0.9^{a}	27.7±0.3^{b}
3	ND+AI	Imuvral	50.0±3.51	44.0±0.58	68.0±1.73^{c}	68.3±6.64^{c}	2.62±0.24	2.8±0.08	3.00±0.17^{bc}	3.0±0.17^{bc}	8.8±0.78	9.2±0.16^{a}	9.4±0.32^{b}	8.7±0.35^{b}	26.3±2.60	27.7±0.33^{a}	28.0±0.5^{b}	26.0±1.1^{b}
4	Control Negative		48.0±0.58	60.0±3.96	90.0±0.0a^{b}	88.0±0.0^{b}	2.93±0.10	2.82±0.05	3.17±0.00^{ab}	3.17±0.0^{ab}	8.3±0.58	8.1±0.40^{b}	10.4±0.0^{a}	8.9±0.09^{b}	25.0±2.08	24.7±1.45^{b}	31.0±0.0^{a}	26.7±0.3^{b}
5	Control AI + ND		56.8±1.45	65.3±5.49	102.7±11.46^{a}	109.3±2.02^{a}	2.58±0.13	2.89±0.32	3.40±0.00^{a}	3.40±0.0^{a}	8.5±0.19	9.4±0.29^{a}	10.8±0.17^{a}	9.6±0.0^{a}	25.0±0.58	28.0±0.58^{a}	32.0±0.5^{a}	30.0±0.0^{a}
LSD 5%					16.4059	10.5345			0.3477	0.3477		0.8699	0.6389	0.6593		2.8955	1.8788	2.1525

- **Each value represents mean ±S.E.**
- **Significant difference between groups by t-student test at P≤ 0.05**
- **#: Significant variation between groups by ANOVA test at P≤ 0.05.**
- **Different superscript letters a, b and c denote significant variation respectively by LSD at P≤ 0.05**

Table (5): Results of differential leucocytes count in immunostimulant treated and vaccinated chicken groups.

Gr. NO	Vaccine	Immune stimulant	Eosinophil				Monocyte				Lymphocyte				Heterophil			
			2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
1	ND + AI	Lector	1.7±0.33	1.3±0.33	1.3±0.33$^{\#b}$	1.0±0.00	4.0±0.58	4.3±0.33$^{\#A}$	2.7±0.33$^{\#A}$	2.3±0.33$^{\#b}$	75.0±2.52$^{\#b}$	81.3±0.88$^{\#a}$	80.3±0.88$^{\#a}$	83.0±0.58$^{\#a}$	19.3±2.40$^{\#B}$	13.0±0.58$^{\#c}$	15.7±0.33$^{\#d}$	13.7±0.33$^{\#d}$
2	ND + AI	Superimmune	1.0±0.58	1.0±0.58	1.3±0.33^{b}	1.0±0.58	2.7±1.20	2.3±0.33^{B}	3.3±0.58^{A}	3.3±0.0^{b}	74.0±1.15^{bc}	83.3±0.88^{a}	78.0±0.58^{b}	80.3±0.33^{b}	22.3±1.45^{Ab}	13.3±0.58^{c}	17.3±0.30^{c}	15.3±0.33^{c}
3	ND + AI	Imuvral	0.3±0.33	0.7±0.33	1.3±0.33^{b}	0.7±0.33	2.7±0.66	3.3±0.33a^{b}	3.3±0.33^{A}	1.3±0.33^{c}	80.3±0.88^{a}	78.0±1.15^{b}	78.7±0.33^{b}	82.7±0.33^{a}	16.7±1.20^{C}	18.0±2.03^{b}	16.7±0.33^{d}	15.3±0.33^{c}
4	Control Negative		1.3±0.33	0.7±0.33	0.0±0.0^{a}	0.0±0.00	4.7±0.33	3.3±0.22^{ab}	5.0±0.0^{B}	6.0±0.0^{a}	70.0±0.58^{c}	73.3±0.33^{c}	71.0±0.0^{c}	70.0±0.0^{d}	24.0±0.58^{A}	22.7±0.33^{a}	24.0±0.0^{a}	24.0±0.00^{a}
5	ControlAI+ ND		1.0±0.58	1.3±0.33	0.7±0.33^{c}	0.7±0.33	3.3±0.33	3.0±0.33^{B}	1.3±0.33^{ab}	1.7±0.33^{c}	74.0±0.58b^{c}	77.0±0.58^{b}	77.7±0.33^{b}	77.3±0.33^{c}	21.7±0.88^{Ab}	18.7±0.33^{b}	20.3±0.33^{b}	20.3±0.33^{b}
LSD 5%					0.9394			1.0503	1.1505	0.8136	4.2532	2.5727	1.6263	1.1494	4.5539	3.1509	0.9394	0.9394

- **Each value represents mean ±S.E.**
- *** Significant differernce between groups by t-student test at P≤ 0.05**
- **#: Significant variation between groups by ANOVA test at P≤ 0.05**
- **Different superscript letters a,b and c denote significant variation respectively by LSD at P≤ 0.05.**

4. Discussion

Newcastle disease is a worldwide problem its Severity depends on the virulence of the infecting virus and host susceptibility, Onset is rapid, and signs appear throughout the flock, Young birds are the most susceptible. Observed signs depend on whether the infecting virus has a predilection for respiratory, digestive, or nervous systems. Respiratory signs, Partial or complete cessation of egg production may occur, it causes direct economic losses for the producer and a potential loss of value in the view of the consumer (Otte and Chilonda 2000). Large numbers of poultry have died from HPAI or been culled to control the disease since it spread widely from 2004 onwards (Otte et al., 2008). Pidotimod, a synthetic immunostimulant, has been shown to improve the responses to inflammatory stimuli acting on different immunological pathways (Ferrario et al. 2015).

In Egypt it was estimated; 36 million poultry have died or culled as a result of HPAI H5N1 (Otte et al., 2008). Mucosal immunity plays an important role in preventing attachment and initial infection of the microbial pathogens. Immunization at the mucosal surfaces produces the protective antibody. The currently used Hitchner B1 and La Sota strains of live virus vaccines are very efficient Passive immunity to NDV is important in young chicks during the first few weeks after hatching. Passive immunity can be effectively established and preserved at an appropriate level by periodic monitoring of maternal antibody titers, because the dam's antibodies are transferred to day-old chicks through egg yolks (Heller et al, 1977). For the primary vaccination of young chickens against viral poultry diseases such as ND and IBD, live vaccines are mostly used. Depending on the antibody titres and the virulence of vaccine viruses, it can be predicted at what age young chicken scan is vaccinated efficiently (Solano et al., 1986).

L-carnitine could improve significantly BW gain only in 35-49 days old ($P<0.05$), but no in earlier ages ($P>0.05$). Also FCR, production index, antibody titers against ND and AI vaccines improved significantly ($P<0.05$). (2) L-carnitine had a little positive improvement on serum components, blood cells count and carcass traits, but they were not considerable and significant statistically ($P>0.05$) (Khoshkhoo et., al 2006). Garlic powder has the potential to increase serum gamma -globulins in broiler chicks vaccinated against common poultry pathogens (Jafari et al 2009). In the current study, compare the effect of different immunostimulants on the immune response of maternally immune broiler chicks given bivelant ND with AI-H5N1 oil vaccine. The Average BW of vaccinated groups with AI+ND given Superimmune at the 2nd weeks is higher (43,94 gm) than other groups , while in case of Imuvral (48.76gm) was higher than both superimmune and lector (48.79 gm each) and control vaccine at the 3rd week. AI-HI antibody titres, at 4th week of using Imuvral was higher than both superimmune and Lector these results were matched with (Li, B., D. Cramer.et al 2007 and Ichinohe, T., A. Ainai et al 2010) as they reported that the β-1,3-D Glucans molecules are potent reticuloendothelial-modulating agents, whose immunobiological activity ismediated by stimulating proinflammatory cytokine production .

ND-HI antibody titres of immunostimulant treated groups were higher than control at 2 and 4th week, as shown in table (2) these results are run nearly with the results of (El-Sayed et al. 2011) as he investigate the effect of different AI vaccines (H5N1, H5N2, combinant AI H5N2+ ND, and Egyptian H5N1) and vaccination programs (at 1 or 7 days-old) on broiler productivity and immunity.

The results of challenge test with vvND strain showed that the control –ve group showed 75% morbidity and 62.5% mortality and in case of Imuvral, Lector and vaccinated groups showed morbidity rate 25%. While in case of Superimmune and control vaccine mortality 12.5 %, as shown in table (3) This finding run hand to hand with those obtained by (Hussein et al. 2009) as he studied the effect of a mixture of some immunostimulant substances on the immune response of broiler chicks to bivalent AI-ND vaccine immune response to the

bivalent AI-ND vaccine. In group 1 the protection reached 100% against the challenge with vNDV and reached 80% in AI-ND vaccined group 2 and 60% in case of live ND vaccined group 3. Bursa and Thymus sections which examined at 4 and 5wpv was apparently healthy in treated groups (plate 1,2). WBCs levels at 4 and 5 wpv, of control vaccined group 5 were significant higher than all immune treated and control –ve groups, RBCs, Hb and PCV of group 5 are higher than control –ve and superimmune groups as shown in the table (4). Differential leucocytes showed higher values of eosinophils in immunostimulants groups than control ones. Monocytes of Lector group at 3 weeks were higher than all groups as shown in table (5). In our study using of beta glucan and lectine and vitamin had a great high effect in WBCs levels specially lymphocytes and monocytes (table 5) and these results relatively matched with (Kong et al. 2004; Zhao et al. 2013). As they repoted that T lymphocyte proliferation is considered an important index to reflect cellular immunity and to evaluate the immune-enhancing activity of immunomodulators In conclusion, results of this study pointed out the importance of immunostimulant usage to obtain higher immune response.

4. Conclusion

The importance of immunostimulant usage to obtain higher immune response during the vaccination against avian influenza and Newcastle viruses as they gave high levels of antibodies and better body performance

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