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Immunomodulating effect of B-glucans and mannan oligosaccharide on broiler chicks vaccinated with Newcastle disease virus

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This study was carried out to determine the immunomodulating effect of β-glucans and mannan oligosaccharide (MOS) on the immune response of chickens to Newcastle disease vaccine. The results showed that birds received β-glucans and MOS having higher average body weights values and significantly higher ND HI antibody titer than the other non medicated groups. Thymus, spleen and bursal indices of control negative showed significantly lower values than vaccinated medicated and non-medicated groups. Both total and differential leukocytic and lymphocytic counts showed significantly higher in medicated group than other groups. Liver function test showed lower AST and ALT in medicated group than other groups. Results of challenge test with NDV confirmed that MOS and B glucans immunostimulant improved protection rate by 15% in medicated than non- medicated ones. In conclusion MOS and B glucans can be given to chicken to improve both body weight and protection against VV NDV challenge that predominated in Egypt.

Commercial poultry flocks receive a lot number of vaccines to protect them from environmental pathogens; therefore, a great effort had been expanded to develop strategies to enhance chicken immune response, especially in facing immunosuppression caused by extraneous agents, infections, intoxication or by certain vaccine viruses. Immunomodulation could improve vaccinal immunity and possibly selectively promote responses that are critical for protection.

Immunomodulators usually classified according to their origin into biological and chemical products (Poli, 1984). This classification further broken down into physiological products, substances of microbial origin and synthesis compounds.

The mannan-oligosaccharide (MOS) is derived from the outer cell wall of yeast, and its evaluation in diets for breeders is of particular interest because it not only shifts gastrointestinal microflora balance toward beneficial organisms (Spring *et al.*, 2000; Fairchild *et al.*, 2001) but also has immunomodulatory properties (Cotter *et al.*, 2002). The yeast cell wall has powerful antigenic stimulating properties, and it is well established that this property is a characteristic of the mannan chain (Ballou, 1970). This study was carried out to determine the immunostimulant effects of commercial feed additive preparations containing a mannan-oligosaccharide plus β-glucans on chicken, performance and immune response to ND vaccine. Body weight gain, HI and challenge With NDV that endemic in Egyptian poultery farms as well as bursal, thymic and spleen body weight ratio were taken as criteria for evaluation based.

Materials and methods

Immunostimulants (ALPHAMUNE®). It's a commercial feed additive product composed of (1-3, 1-6) β-glucans and (MOS) obtained from Alpharma Animal Health. USA (patch NO AG51242).It was used in ration at a rate of 500 gram/ ton of finished fed.

Chickes. A total number of 225 one day-old commercial (white HI-line rooster) chickens obtained from El-Wady Company were used in this study.

Newcastle disease (ND) vaccinal strains. I-Hitchiner B1 and La Sota strains, produced by Pfizer International Company, USA with each vial contain virus titre of 10^9 EID_{50} was used after titration for vaccination of experimental chicks via eye instillation route.

Clone 30. Vaccine nobilis clone 30 (Lot No: 06829AJ01, Intervet international B.V. Boxmeer – Holland) with virus titer of 10^6 EID_{50} was used for vaccination of experimental chicks via eye instillation

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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 were used for challenge test.

Haemagglutination (HA) and haemagglutination inhibition (HI) tests. HA and HI test were carried out according to (Anon. 1971).

Haemtological studies. Total leukocytic count was counted according to the technique described by (Nutt and Herrick, 1952) while differential leukocytic count was done by the standard method of Battelnent described (Schalm, 1973).

Biochemical analysis. ALT and AST were done according to the method of (Reitman and Frankel, 1957). Serum uric acid was done according to the method descried by (Barham and Trinder, 1972) and serum creatinin was done according to the method described by (Houot, 1985).

Bursa body weight index. It was calculated according to (Ying *et al.*, 2003) as following: Bursa: body weight ratio = bursa weight/ body weight. Bursal index = Bursa: body weight ratio X 1000.

Challenge test. Chickens were challenged via intramuscular route. Each chicken received a dose of 0.5ml / bird containing 10^6 EID_{50} VV NDV according to (Afify, 1990). Birds with persisted symptoms till the end of the observation period were considered as if dead.

Statistical analysis. Statistical analysis of variance (ANOVA) test was used to estimate differences among treatments according to (Steel and Torrie, 1960).

Experimental design. The used chicks (225) were floor reared and fed on balanced commercial ration free from antimicrobial agents. At the 1st day of life, 5 chicks were sacrificed for organ body weight ratio and serum while the rest of obtained chicks were divided into 4 groups (1-4). Groups (1 and 3) containing 60 chicks each, while groups 2 and 4 containing 50 chicks each. Each group was kept in a separate clean disinfected room.

Chicks of groups 1 and 3 were feed on ration without additives, while those of groups 2 and 4 were feed on ration supplemented with the immunostimulant (Alphamune®), in dose of 0.5 gm/ kg. At the 5th day of age all chicks were S/C vaccinated with inactivated avian influenza (H5N1) (0.3 ml/ bird). At the 7th day of age, chicks of group (1 and 2) were kept ND non-vaccinated control while birds of groups (3 and 4) were vaccinated each with 10^6 EID_{50} Hitchiner B1 via eye instillation and revaccinated at the 18th day of age as each bird was given 10^6 EID_{50} La Sota via eye instillation route. At 35 day of age 20 birds from groups were separated and challenged with 0.5 ml containing 10^6 VVND. Challenged chickens were kept under daily observation for 21 days with daily record of symptoms, deaths and post – mortem lesions. Ten birds from group 1 and 3 were left without challenge to be control.

Experimental chicken groups were weekly subjected to the following: Life body weight of random 5 birds / group as well as weight of bursal, thymus and spleen of each bird was recorded to calculate organ body weight ratio. Random 5 non-coagulated blood samples on EDTA were collected for total anddifferential leukocytic count. Random coagulated 5 blood samples from wing vein were collected for serum collection. The collected serum samples were divided into two equal quantities, labeled and stored at-20°C until use. The collected sera were tested for detection of NDV HI as well as liver and kidney function test.

Results and Discussion

There are large numbers of immunostimulatory components were reported to be used for stimulating the chicken immune response to face the problem of vaccination failure, which constitute a challenge to poultry industry all over the world. The application of immunostimulant is not only to raise resistance of birds but also to improve their immune response to vaccination (Afify, 1990; Awaad *et al.*, 2000). The work was designed to evaluate the effect of Alphamune as immunomodulator in chickens, where data presented in (Table 1) showed that administration of Alphamune was significantly increased body weight at 7 days old 80.30±1.88 gm verses 72.90 ±1.58 for control group. While from 14 to 35 days there is no significant difference could be detected values of different groups as well as that of control group. This result agrees with those of Solis de los (Santos *et al.*, 2007) where a significant weight difference at 7 days only between treated and non treated poults was found while no difference at 3 weeks old.

Table (1): Effect of immunostimulant on average body weight of ND vaccinated and non- vaccinated chickens.

Group No.	Treatment I.S	Treatment Vacc	Age / week 0	1	2	3	4	5
1	-	-	36.39±1.2	72.90±1.58	130.00±2.31	221.80±8.40	319.30±4.29	428.40±9.93
2	+	-		80.30±1.88*	133.00±3.21	228.50±5.5	332.00±5.09	446.25±17.95
3	-	+			135.40±2.85	225.00±4.18	325.60±5.26	428.60±15.93
4	+	+			137.60±2.35	229.60±5.28	343.50±12.04	467.50±14.16

Each value represents mean ±S.E.
* Significant difference between groups by t-student test at P≤ 0.05.

Table (2): The effect of immunostimulant on mean ND HI antibody titer in vaccinated and non-vaccinated chickens.

Group No.	Treatment I.S	Treatment Vacc	Age / week 0	1	2	3	4	5
1	-	-	7.8±86	6 ± 0.32	# 4 ± 0.55 b	# 2.4±0.51b	# 1.6±0.51b	# 1.6±0.51b
2	+	-		6.2±0.37	4.4± 0.51b	2.8± 0.37b	1.8± 0.37b	1.8± 0.55b
3	-	+		6± 0.32	6.4± 0.51a	7.2± 0.58a	6.6± 0.69a	5.6± 04a
4	+	+		6.2±0.37	7.2±0.37a	7.8± 0.58a	7.2± 0.37a	6.2± 0.49a

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

Table (3): Effect of immunostimulant on thymus index of vaccinated and non- vaccinated chickens with NDV live vaccine.

Group No.	Treatment I.S	Treatment Vacc.	Age / week 0	1	2	3	4	5
1	-	-	4.60±0.12	5.60±0.06	#6.00±0.10b	#6.10±0.08b	#6.70±0.12c	#5.60±0.18c
2	+	-		5.9±0.07*	6.60±0.09a	7.21±0.15a	7.65±0.23b	5.80±0.20b
3	-	+			6.63±.0.11a	7.25±0.20a	7.91±0.22ab	6.33±0.21ab
4	+	+			6.71±0.10a	7.50±0.2a	8.35±0.30a	6.50±0.25a

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 (4): Effect of immunostimulant on mean spleen index of vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment I.S	Treatment Vacc.	Age / week 0	1	2	3	4	5
1	-	-	0.50±0.01	1.00±0.05	#1.50±0.08c	#1.90±0.10c	#2.05±0.08c	2.30±0.05
2	+	-		1.35±0.07*	1.70±0.07c	2.10±0.10bc	2.10±0.15c	2.34±0.06
3	-	+			2.30±0.11b	2.46±0.12b	2.50±0.11b	2.40±0.09
4	+	+			2.63±0.15a	2.87±0.17a	2.90±0.18a	2.44±0.15

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): Effect of immunostimulant on mean bursal index of vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment I.S	Treatment Vacc.	Age / week 0	1	2	3	4	5
1	-	-	1.80±0.08	2.50±0.10	#2.80±0.15c	#3.20±0.12b	#2.41±0.15c	#1.55±0.11b
2	+	-		2.85±0.12*	2.90±0.14c	3.40±0.12b	2.60±0.13bc	1.85±0.12b
3	-	+			3.20±0.13b	3.80±0.14ab	3.00±0.19b	2.50±0.15a
4	+	+			3.43±0.13a	4.10±0.18a	3.55±0.21a	2.75±0.20a

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 (6): Effect of immunostimulant on mean total leucocytic count X 10^3 of vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment		Age / week					
	I.S	Vacc.	0	1	2	3	4	5
1	-	-	16.1±1.30	14.3±1.10	#11.6±0.50b	#12.1±0.74b	#13.1±0.51b	#13.2±0.9b
2	+	-		22.5±1.30**	17.3±1.30ab	15.6±0.75ab	14.8±0.8ab	13.4±0.95b
3	-	+			20.6±1.15a	21.5±1.20a	22.2±1.23a	21.1±1.25b
4	+	+			26.2±1.20a	28.5±1.9a	32.3±1.7a	30.6±2.3a

Each value represents mean ±S.E.
** Significant difference between groups by t-student test at P≤ 0.01.
#: 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.

Regarding thymus index, spleen index and bursal index of chicken fed on Alphamune supplemented ration and vaccinated with NDV revaled significant increase in values than results of other groups where it give 6.50 ± 0.25, 2.44 ± 0.15 and 2.75 ± 0.20 respectively at 35 days of age (Table 3-5). These results come in agreement with the finding of Ying *et al.*, (2003) where mean percentage of organ body weight ratios of liver, spleen, Kidney, thymus and bursa of Fabricius exhibited a significant (P<0.05) increase in MOS as compared to those of control group. Results of total and differential leucocytic count (Table 6, 7) were significantly higher TLC on group 4 at 35 days of age (30.6 ± 2.3) in comparison to (13.2 ± 0.9, 13.4 ± 0.95, 21.1 ± 1.5) for the other groups. It was observed that the source of increased in TLC is the significantly increased lymphocyte counts due to use of Alphamune®, Increased TLC in group 4 can be attributed to immunostimulation effect of compounds of Alphamune. This result was previously observed by (Fleischer *et al.*, 2000; Acevedo *et al.*, 2001) who recoded increased TLC with administration of MOS and b-glucan respectively. Chicken group 4 that fed on Alphamune® supplemented ration and vaccinated with NDV vaccine showed significant lower AST and ALT levels at 35 days of age (Table 8, 9). Where the results is 172.17 ± 7.15 and 9.85 ± 0.20 respectively verses 201.56 ± 7.53 and 14.3 ± 0.25 in untreated vaccinated group .This result was observed by (Santhosh *et al.*, 2003) in treated group with MOS all over the breeding period.

Statistical analysis of uric acid and creatinine values (Table 10, 11) resulted in non significant difference between different groups up to 35 days of age. The instability in creatinine value from week to week may be related to change in feed and protein concentration. Birds of group (4) showed significant HI titers to NDV at 35 days of age than other groups (Table 2); this higher HI titers resulted in 95% protection in this group 4 compared to 85% protection in group 3 , 20% protection in group 2 and 0% protection in group 1 (Table 13). Our results clearly showed the specific immune stimulation and protection against challenge in group 4 were attributed to B-glucan compound of Alphamune due to increasing functional activity of macrophage and neutrophils. Yun *et al.*, (2003); Sakurai *et al.*, (1992) reported that orally B-glucan indirectly stimulate the immunity in the respiratory system of mice by activating macrophage in the payer's patches of the gut.

From the above discussed data we could conclude that Alphamune® could increase body weight gain, improve immunity of the birds and decrease susceptibility to NDV challenge.

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Table (7): Mean differential leucocytic count in immunomodulator medicated vaccinated or non-vaccinated chickens with NDV live vaccine.

Age / week	Group No. and treatment																
	Group 1: Control				Group 2: I.S				Group 3: vaccinated				Group 4: I.s +vaccination				
	H	L	M	E	H	L	M	E	H	L	M	E	H	L	M	E	
0	20.5	74.7	2.9	1.9	20.5	74.7	2.9	1.9	20.5	74.7	2.9	1.9	20.5	74.7	2.9	1.9	
1	25.8*±1.08	67.8±1.07	3.6±0.24	2.8±0.2	17.2±0.58	77.8*±0.37	3±0.32	2±0.32	25.8*±1.08	67.8±1.07	3.6±0.24	2.8±0.2	17.2±0.58	77.8*±0.37	3±0.32	2±0.32	
2	23.8±1.02	73.4±0.75	2.0±0.32	1.2±0.37	20.4±0.51	75.6*±0.4	2.2±0.37	1.8±0.2	45.8*±0.37	48.0±0.89	3.4*±0.24	2.8*±0.37	41.6*±0.51	52.1±0.32	2.7±0.37	3.6*±0.4	
3	20.4±0.81	76.4*±0.51	2.6±0.24	1.2±0.58	18.2±0.37	77.2*±0.37	3.4±0.6	0.6±0.24	49.8*±0.37	43.6±0.24	2.8±0.37	4.0*±0.2	44.8*±0.37	49.6±0.24	2.2±0.37	3.4*±0.24	
4	17.6±0.51	76.8*±0.37	2.8±0.51	2.8±0.37	18.4±0.51	77.6*±0.51	1.8±0.2	2.2±0.37	46.6*±0.75	48.6±0.51	2.2±0.37	2.6±0.24	34.1*±0.32	59.5*±0.52	2.2±0.37	2.6±0.24	
5	20.4±0.93	74.4±0.93	1.8±0.2	2.4±0.24	17.2±0.86	77.6*±1.12	2.4±0.37	2.8±0.37	42.0*±0.71	52.2±0.58	1.8±0.2	3.6±0.37	33.6*±0.57	61.0*±0.71	1.8±0.2	3.4±0.4	

*Significant difference at P≤ 0.05 between treated and non treated groups.
*H = Heterophils; L = Lymphocytes; M = Monocytes; E = Eosinophils.

Table (8): Effect of immunostimulant onmean of AST in sera of vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment		Age / week					
	I.S	Vacc.	0	1	2	3	4	5
1	-	-	101.30±4.15	131.30±6.21	#155.00±5.90 a	#162.30±5.35 a	#174.55±5.84 b	#190.10±6.82 a
2	+	-		125.32±5.85	130.10±6.15 b	142.60±5.60 b	159.21±6.17 b	168.32±6.25 b
3	-	+			160.30±7.51 a	171.25±5.85 a	194.80±8.13 a	201.56±7.53 a
4	+	+			136.50±6.22 b	139.50±5.51 b	162.23±6.54 b	172.17±7.15 ab

Each value represents mean ±S.E.
#: 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 (9): Effect of immunostimulant on mean of ALT in vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment		Age / week					
	I.S	Vacc.	0	1	2	3	4	5
1	-	-	4.92±0.15	7.90±0.20	#10.17±0.20 a	#12.77±0.22 a	#13.12±0.25 b	# 13.50±0.26 b
2	+	-		7.76±0.21	8.65±0.22 b	11.20±0.25 b	10.57±0.22 c	12.30±0.26 c
3	-	+			10.50±0.21a	12.89±0.26 a	14.05±0.27 a	14.30±0.25 a
4	+	+			8.10±0.18 b	9.60±0.20 c	9.70±0.21 d	9.85±0.20 d

Table (10): Effect of immunostimulant on uric acid in sera of vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment		Age / week					
	I.S	Vacc.	0	1	2	3	4	5
1	-	-	5.30±0.13	6.10±0.15	6.30±0.12	6.42±0.20	6.20±0.21	5.90±0.20
2	+	-		6.21±0.14	6.20±0.13	6.40±0.18	6.18±0.25	6.00±0.22
3	-	+			6.50±0.17	6.48±0.19	6.51±0.23	6.25±0.20
4	+	+			6.40±0.16	6.41±0.20	6.48±0.25	6.20±1.54

Table (11): Effect of immunostimulant on creatinine level in sera of vaccinated and non-vaccinated chickens with NDV live vaccine.

Group No.	Treatment		Age / week					
	I.S	Vacc.	0	1	2	3	4	5
1	-	-	1.12±0.10	1.05±0.03	1.15±0.03	1.25±0.04	1.30±0.06	1.28±0.04
2	+	-		1.10±0.04	1.10±0.04	1.20±0.04	1.26±0.03	1.30±0.04
3	-	+			1.21±0.05	1.25±0.05	1.29±0.05	1.40±0.05
4	+	+			1.20±0.03	1.23±0.05	1.31±0.06	1.34±0.06

Table (12): Daily distribution of morbidity and mortality in challenged chickens.

Group No.	Treatment		Observation	Days post-challenge										Total	%
	I.S	Vacc.		1	2	3	4	5	6	7	8	9	10	11-21		
1	-	-	Diseased No				5	7	4	2	1	-	-	-	19	95
			Died No.			3	4	5	5	2	1	-	-	-	20	100
2	+	-	Diseased No				2	3	5	3	2	2	1	-	18	90
			Died No.				1	3	4	5	2	1	1	-	17	85
3	-	+	Diseased No				1	1	2	1	1	-	-	-	6	30
			Died No.					1	2	-	-	-	-	-	3	15
4	+	+	Diseased No					1	1	1	1	-	-	-	4	20
			Died No.						1	-	-	-	-	-	1	5

Table (13): Results of VVND challenge test in immunostimulant medicated on vaccinated and non-vaccinated chickens.

Group No	Treatment		Total No of birds	No of dead birds	No of survived birds	Protection %
	I.S	Vacc.				
1	-	-	20	20	0	0
2	+	-	20	17	3	15
3	-	+	20	3	17	85
4	+	+	20	1	19	95

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التأثير المناعي لمادة البيتا جلوكانز والمانان اوليجو سكاريدز على الكتاكيت المحصنة بلقاح النيوكاسل

تم دراسة التأثير المناعي لمادة البيتا جلوكانز والمانان اوليجو سكاريدزفي الكتاكيت المحصنة بلقاح النيوكاسل وأظهرت النتائج أن الكتاكيت التي تم معاملتها بهذه المعاملات أعطت معدل أعلى في أوزان الجسم والغدة التيموسية والطحال وغدة فابريشيس كما أظهرت المجموعة المعاملة بهذه المواد مستوى أعلى في الكم والنوع في كرات الدم البيضاء وأظهرت القياسات للمجموعة المعاملة مستوى اقل من المجموعات الاخرى في إنزيمات الكبد كما أظهرت النتائج ارتفاع مستوى الأجسام المناعية للقاح النيوكاسل في هذه المجموعة مما أعطى معدل حماية اعلي في اختبارا لتحدى. من النتائج يمكننا أن نستنتج أن استخدام البيتا جلوكانز والمانان يؤدى إلى تحسن في الأوزان وكذا معدلات الصد ضد عدوى التحدي بفيروس النيوكاسل شديد الضراوة والمنتشر في مصر.