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Short Communication

Immunomodulatory Effect of Polyimmune (*Astragalus membranaceus*) Extract on Humoral Response of Layer Birds Vaccinated against Newcastle Disease Virus

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ABSTRACT

Immunomodulatory effect of a commercially available phytic polysaccharide product with the name Polyimmune (*Astragalus membranaceus* extract) was determined on the humoral response of Newcastle disease (ND) virus vaccine and subsequent immunosuppression caused by infectious bursal disease (IBD) vaccine in layer birds. In this study one hundred days-old layer chicks were purchased and divided into five groups namely A, B, C, D and E (20 chicks in each group) and inoculated with various combinations of vaccines with or without substitution of polyimmune. At the age of 33 days all the birds were primed with NDV vaccine and then after 12 days post priming, all the birds were given booster dose of same NDV vaccine. Similarly 7 days post boosting the birds were re-vaccinated against IBDV except group E. Whereas, groups A, B and C were substituted with varying dosage of polyimmune (for three consecutive days). Serum samples were collected at the age of 30, 45, 53, 61, 69 and 77 days of age. Haemagglutination inhibition (HI) titers of these serum samples against NDV were analyzed and the geometric mean titer of groups A, B, C, D and E as per above-mention sequence of days except for day 30, where all groups were having GMT 2.1, rest were 6.34, 7.23, 6.44, 6.10 and 7.10; 7.21, 7.34, 7.24, 7.41 and 7.31; 6.35, 7.35, 7.38, 5.46 and 7.32; 6.27, 7.41, 7.45, 3.67 and 7.21; 5.90, 7.10, 7.16, 3.40 and 6.92, respectively. The result obtained revealed that IBD have adverse effects on ND vaccine but the recommended dosage of polyimmune has immunostimulatory effect. However, varying dosage has non-significant difference in compensating humoral response than normal dosage.

Key Words: Polyimmune; Newcastle disease; Infectious bursal disease and vaccine

INTRODUCTION

Newcastle disease (ND) vaccination is routine in commercial chicken flocks in most Asian countries. The programmes and procedures differ between countries and even between farms in the same country, depending on local circumstances (Aini, 1990). Despite vaccination, outbreaks are not un-common. This may be due to vaccine failure, immunosuppression caused by mycotoxins, other live vaccine, environmental and managemental stress. In layer ND and infectious bursal disease (IBD) are very important diseases but the immunosuppressive effect of latter had previously been reported to adversely affecting the vaccination against ND (Allan *et al.,* 1972). Proper vaccination program and strict biosecurity measures along with administration of immune booster were proved in many reports as essential tool for the control of the diseases (Giambrone & Clay, 1986; Wyeth & Chettle, 1990; Haddad *et al.*, 1997).

Polyimmune (*Astragalus Polysaccharide*) is a phytic product that contains *flavonoids, saponins, polysaccharide (D-glucose*), *glucids* and *phytosterols*. Investigative studies have shown a wide range of immunostimulating effects of polyimmune. Pharmacologically, polyimmune along with watery extracts or fractions enriched with Saponins or polysaccharide has shown hepatoprotective (Wang *et al.,* 2001) affect by decreasing carbon tetrachloride; act as antioxidant by inhibiting lipid peroxidation (Zhang *et al*., 2003). It also has immunostimulant, antiviral and cardio tonic properties (Duan *et al.*, 2002; Yang *et al*., 2001; Wang *et al.,* 2002). The polyimmune has stimulating properties without suppressing the immune function as the glucid fraction stimulates the Natural killer cells and increase the function of T-cells and production of Interferon. It improves the cytotoxic activity of specialized cells five to six times (Xue *et al.,* 1999). Polyimmune enhances the phagocytosis and natural interferon production (Wang *et al.*, 2002). This project was designed to study the efficacy of varying dosage of a commercial product (polyimmune) in terms of its Immunomodulatory effect using as ND and IBD vaccine as model.

MATERIALS AND METHODS

Experimental birds. One hundred layer day old chicks were purchased and reared in the experimental poultry

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house of Department of Microbiology, University of Veterinary and Animal sciences Lahore. At the age of one month the birds were divided into five groups, namely A, B, C, D and E having 20 heads per group. All the Groups were vaccinated against ND and IBD vaccines, while Group A, B and C were injected intramuscularly with varying dosage (0.5, 1 & 2 mL) of Polyimmune, respectively. Group D and E served as polyimmune negative control and ND vaccine control, respectively (Table I). Chicks were primed with ND live vaccine (Lasota, Fort Dodge) intraocularly, at the age of 33 days. Twelve days post-priming, birds were boosted with same ND live vaccine intraocularly. Seven days post ND boosting the birds was vaccinated intraocularly with IBD along with Polyimmune intramuscularly at the dose rate of 0.5 mL, 1 mL and 2 mL for three consecutive days.

Haemagglutination inhibition test (HI). Sera were procured from ten chicks of each group randomly at the age of 30, 45, 53, 61and 69 days and stored at -20ºC till further analysis. HI test was performed as described by Allan *et al.* (1978). Fifty micro liters of serum was serially diluted (Two fold) with normal saline in V shaped micro titer plates up to well No 10. Fifty micro liters of 4 HA units of ND virus were added up to well No 11, which served as virus control. Twelveth well was the control for reagents (it only contain RBCs & normal saline). The plate was shaken and left for 30 min at room temperature allowing antigen and antibody to react. After that 50 µL of 1% washed chicken RBCs were added in each well of the plate and left for 20 min. The HI titer was expressed as the reciprocal of the highest dilution that causes inhibition of agglutination and geometric mean titer (GMT) was calculated.

The collected data was analyzed statistically by least significant difference test (LSD) to observe whether varying dosage of polyimmune has significant effect or not in potentiating the humoral response against ND after administering IBD vaccine.

RESULTS AND DISCUSSION

Both Newcastle disease (ND) and infectious bursal disease (IBD) causes huge economic losses to poultry industry in many parts of the world. In Pakistan, number of vaccines had been introduced to control these maladies with failures encountered from time to time. The salient feature analyzed in this study was to determine the role of polyimmune (natural product) on Newcastle Disease vaccinal titer and immunosuppression caused by Infectious Bursal Disease.

The GMT of randomly taken sera before ND vaccination was found to be 2.1, which showed the presence of maternally derived antibodies (MDAs), as the eggs were laid by the hens with history of Newcastle Disease vaccination, which is in agreement with previous findings (Allan *et al.*, 1978). These MDAs are found to protect against previously proved pathogenic effects in vaccinated chicks by ND Lasota vaccine (Murphy *et al*., 1999). This is the reason that no appreciable morbidity and mortality was recorded. The birds were primed with ND vaccine at the age of 33 and calculated titers were found to be protective. Seven days post boosting resulted in further increase in antibody titer. The GMT following vaccination against the IBD and Polyimmune for groups B and C yielded a sustained level of antibodies against ND on 7, 14 and 21 days post vaccination whereas group A showed slight decrease in titer with the passage of time. However, post vaccination with IBD in group D showed a rapid decline in antibodies against ND, where no polyimmune was administered (Table I).

The data collected was analyzed statistically by least significant difference test (LSD), which indicated significant difference ($p<0.05$) in antibody titer of the group A as compared to B and C (Table II) treated with varying dosage of polyimmune, while non significant difference was recorded between B and C and vice versa (Fig. 1).

The immunostimulatory effect of polyimmune on the humoral immune response could be due to the fact that Polyimmune stimulates immune system, anti-tumor activity (Wang *et al.*, 2002) and megakaryocyte colony stimulating activity (Zhu and Zhu, 2001). The natural products affect the immune system of different species in interesting and

Table I. GMT of groups at different stages of treatment

Groups*	GMT** calculated on					
	30 days	45 days	53 days	61 days	69 days	77 days
A	2.1	6.34	7.21	6.35	6.27	5.90
B		7.23	7.34	7.35	7.41	7.10
C		6.44	7.24	7.38	7.45	7.16
D		6.10	7.41	5.46	3.67	3.40
E		7.10	7.31	7.32	7.24	6.92

* = A= ND + IBD + Polyimmune 0.5ml/bird
B= ND + IBD + Polyimmune 1ml/bird
C= ND + IBD + Polyimmune 2ml/bird
D= ND + IBD
E= ND
** = GMT = Geometric mean titer
ND and IBD vaccines were given intra- ocularly and polyimmune was given intramuscularly

Fig. 1. Effect of varying dosage of polyimmune on HI titre of treated group

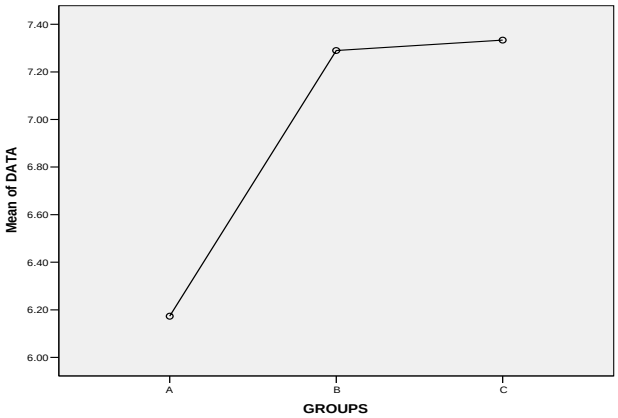

Table II. Least significant difference test of groups A, B and C multiple comparisons

Groups (I)	Groups (J)	Mean difference (I-J)	Standard error	Significance	95% Confidence Interval	
					Lower bound	Upper bound
A	**B**	-1.11667(*)	0.15639	0.000	-1.4994	-0.7340
	C	-1.16000(*)	0.15639	0.000	-1.5427	-0.7773
B	**A**	1.11667(*)	0.15639	0.000	0.7340	1.4994
	C	-0.04333	0.15639	0.791	-0.4260	0.3394
C	**A**	1.16000(*)	0.15639	0.000	0.7773	1.5427
	B	0.04333	0.15639	0.791	-0.3394	0.4260

Dependent Variable: DATA LSD

* The mean difference is significant at the .05 level

complicated way. However, the direct effect might be related to stimulating the lymphatic tissue; proliferative activity of peripheral blood mononuclear cells and promotes I gG production by peripheral blood B-cell (Wang *et al.*, 2002). Polyimmune strengthens the tumor cell phagocytosis and cytokines like TNF-alpha and IL-6 (Wang *et al.*, 2002) that promote B-cell proliferation and then they are changed to plasma cells, which would be able to produce antibodies. On the other hand, polyimmune have anti-oxidant and anti-inflammatory (Zhang *et al.*, 2003) effects that causes inhibition of prostaglandin synthesis as an anti-immune substance and resulting better humoral response. Moreover, the experiment also revealed that increasing dosage than normal has no significant effect on the development of antibody titer. (Table I).

CONCLUSION

The study revealed that Polyimmune not only serves to combat the adverse effects of IBD on ND vaccinal titer but also improves the response of birds to ND vaccine. Moreover, it was concluded that recommended dosage of polyimmune has satisfactory effects on ND vaccinal titer as compared to increasing the dosage. However, decreasing the dosage than recommended does not produce satisfactory results.

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