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RESEARCH ARTICLE

EVALUATION OF IMMUNOPOTENTIATING EFFECT OF MEDICINAL PLANT PRODUCTS IN COMMERCIAL LAYER FLOCK VACCINATED AGAINST NEWCASTLE DISEASE BY HAEMAGGLUTINATION INHIBITION (HI) TEST

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ABSTRACT

The present study was undertaken to evaluate the immunopotentiating effect of medicinal plant products such as *Withania somnifera*, *Tinospora cordifolia*, *Allium sativum* and *Azadirachta indica* in commercial layer flock vaccinated against Newcastle disease. The HI titre values in all the groups were above the protective level throughout the study period. *Withania somnifera* treated group showed highest mean HI titre of 234.66 and high HI titre from 26th week to 40th week of age when compared with other groups. For normal egg production, HI titre value should be greater than 256, which is obtained in *W. somnifera*, *T. cordifolia* and *A. sativum* treated groups only at 32 weeks of age.

INTRODUCTION

India is the fifth largest producer of eggs and ninth largest producer of poultry meat in the world, producing over 34 billion eggs and about 600,000 tonnes of poultry meat. Over the past decade the poultry industry in India has contributed approximately 100 billion rupees to the Gross National Product (GNP) (**Vetrivel and Kumarmangalam, 2010**). Poultry industry is recognized as an important cottage as well as fast growing large commercial agriculture industry with annual growth rate of 12 to 15 per cent. Nowadays, traditional plant products are used in livestock and poultry for treatment, alleviation of stressful conditions and enhancement of disease resistance through its immunomodulatory effects. Plant products have been attracting medical attention for their effective and amazing cures for thousands of years and today these are the most widely used medicines in the world (**Sarwar *et al*., 2011**). Hence, the present study was aimed with the Assessment of immunopotentiating effect of *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Gulancha), *Allium sativum* (Garlic) and *Azadirachta indica* (Neem) in commercial layer flock vaccinated against Newcastle disease by Haemagglutination inhibition test.

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Review of Literature

Newcastle disease (ND) is a highly contagious and fatal viral disease of poultry. It is characterised by diarrhoea, prostration, oedema of the head and wattles, paralysis, torticollis, respiratory distress, decline in egg production, perhaps leading to complete cessation of egg laying, may precede more overt signs of disease and deaths in egg-laying birds (**Alexander, 2000a**). It is an economically important poultry disease in many developing countries, the virulent form of disease is endemic and therefore represents an important limiting factor in the development of commercial poultry production (**Alexander and Senne, 2008**). Newcastle disease was first recorded by Edwards in July 1927 in a place called Ranikhet (Uttar Pradesh) situated in the Kumaon Foot Hills of Himalayas (**Cooper, 1931**) after that the disease was reported from all parts of India (**Haddow, 1941**). In Tamil Nadu, the disease was first reported by **Kylasamaier (1931). Roy and Venugopalan (1997)** reported the incidence of ND in organised layer farms in Tamil Nadu.

Haemagglutination inhibition (HI) test

Haemagglutination inhibition test is the most convenient, rapid, economical method for evaluating the immunity of chickens and turkeys to ND (**Beard and Max Brugh, 1975**).

Maldonado *et al.* (1992) utilized HI test to study the prevalence of antibodies to avian paramyxo virus serotypes 1,2 and 3 in wild and domestic birds. Micro HI test were used to measure the level of maternal IgG in the tears of chicks and also to measure the levels of HI antibodies in the tears and serum after vaccination with 'F' strain of NDV (**Elankumaran *et al*., 1996**). The HI test is still the most widely used conventional serological method for measuring anti-NDV antibody levels in poultry sera, and it is considered as the standard laboratory test for this disease (**Xu *et al*., 1997**). However, sera from other species tend to give a high incidence of false-positive results to ND in HI test and although the number of nonspecific agglutination reactions can be reduced by pretreatment with heat and kaolin but these procedures decrease the sensitivity of this test (**Williams *et al*.,1997**).

MATERIALS AND METHODS

Newcastle disease virus

The freeze dried Ranikhet disease virus (LaSota strain) obtained from Ventri Biologicals, Pune was used as the source of NDV antigen.

Standard positive Newcastle disease serum

The ND standard positive serum obtained from Avian Disease Diagnosis and Surveillance Laboratory, Namakkal was used as standard positive control serum in the HI test.

Standard negative control serum

Standard negative control serum was obtained from birds unvaccinated against Newcastle disease which were maintained at commercial layer farm in Thindamangalam, Namakkal.

Methods

The experimental layer birds were divided into five groups viz., A, B, C, D and E and each group comprises of 24 birds (four replicates in each group) and reared at the commercial poultry farm, Thindamangalam and were raised on caged system.

Table 1. Experimental groups

Groups	Number of birds	Supplementation	Level of inclusion
A	24	*Withania somnifera*	1.0% w/w
B	24	*Tinospora cordifolia*	1.0% w/w
C	24	*Allium sativum*	0.3% w/w
D	24	*Azadirachta indica*	0.2% w/w
E	24	Untreated control	-

Vaccination schedule

Table 2. Vaccination schedule

Age of vaccination	Strain used	Route of administration
5th day	F1	Eye drops
28th day	La Sota	Eye drops
56th day	RDVK	subcutaneous
20th week	R_2B	Intramuscular
25th week	R_2B	Intramuscular

Haemagglutination Inhibition (HI) test procedure

The test was conducted as per the method described by OIE (2009). Ranikhet disease virus LaSota was used as the HA antigen.

This test system included the following controls

1. PBS control : 0.05 ml
2. Serum control : 0.025 ml and 0.025 ml saline
3. Virus control was kept as back titration in 0.05 ml aliquots containing 4, 2, 1, 0.5 and 0.25 HA units. To the above samples 0.05 ml of 1 per cent chicken erythrocytes were added and the plates were incubated at 37^{0}C for 45 minutes before the results were read. The reciprocal of the highest dilution of the serum having complete haemagglutination inhibition of virus was taken as the HI titre in 0.025 ml of the serum.

RESULTS

Assessment of humoral immune response by HI test

The humoral immune response of birds fed with medicinal plant products was assessed by HI test at fortnight intervals from 20 to 40 weeks are shown in Table 1, Fig. 1 and Plate 3.
1.The result shows that all birds under the trial have responded to R_2B vaccination given at 20th and 25th weeks of age and the titre values gradually increased from 20th to 40th week of age.

Table 1. Assessment of humoral immune response by hi test *- (P<0.05), ** - (P<0.01)

Group	Titre	Age of the birds (weeks)											Overall
		20	22	24	26	28	30	32	34	36	38	40	
A	MEAN	85.33	106.66	85.33	170.66	213.33	213.33	256.00	426.66	341.33	341.33	341.33	234.66
	GMT	80.63	101.59	80.63	161.26	203.18	203.18	256.00	406.37	322.53	322.53	322.53	204.53
	Log_2value	6.33*	6.67*	6.33*	7.33*	7.67*	7.67*	8.00**	8.67*	8.33*	8.33*	8.33*	7.61**
B	MEAN	53.33	85.33	149.33	170.66	128.00	170.66	256.00	341.33	341.33	341.33	256.00	208.48
	GMT	50.79	80.63	128.00	161.26	128.00	161.26	256.00	322.53	322.53	322.53	256.00	199.05
	Log_2value	5.67*	6.33*	7.00*	7.33*	7.00*	7.33*	8.00**	8.33*	8.33*	8.33*	8.00*	7.42**
C	MEAN	53.33	85.33	106.66	128.00	170.66	170.66	256.00	341.33	341.33	341.33	341.33	212.36
	GMT	50.79	80.63	101.59	128.00	161.26	161.26	256.00	322.53	322.53	322.53	322.53	202.69
	Log_2value	5.67*	6.33*	6.67*	7.00*	7.33*	7.33*	8.00**	8.33*	8.33*	8.33*	8.33*	7.42**
D	MEAN	85.33	149.33	170.66	128.00	128.00	170.66	170.66	213.33	341.33	256.00	341.33	195.87
	GMT	80.63	128.00	161.26	128.00	128.00	161.26	161.26	203.18	322.53	256.00	322.53	186.60
	Log_2value	6.33*	7.00*	7.33*	7.00*	7.00*	7.33*	7.33**	7.67*	8.33*	8.00*	8.33*	7.42**
E	MEAN	106.66	53.33	53.33	106.66	149.33	106.66	106.66	149.33	85.33	106.66	106.66	102.78
	GMT	101.59	50.79	50.79	101.59	128.00	101.59	101.59	128.00	80.63	101.59	101.59	95.25
	Log_2value	6.67*	5.67*	5.67*	6.67*	7.00*	6.67*	6.67**	7.00*	6.33*	6.67*	6.67*	6.52**

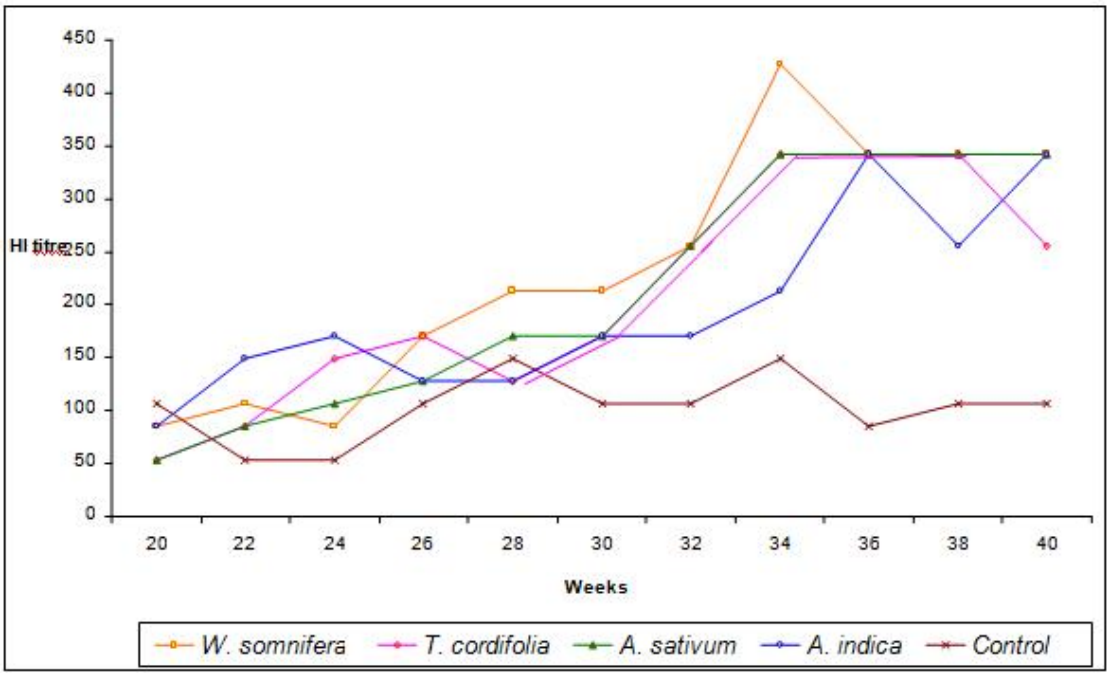

Fig. 1. Assessment of humoral immune response by HI test

Plate 3 Assessment of humoral immune response by HI test

Plate 3a Conduct of HI test

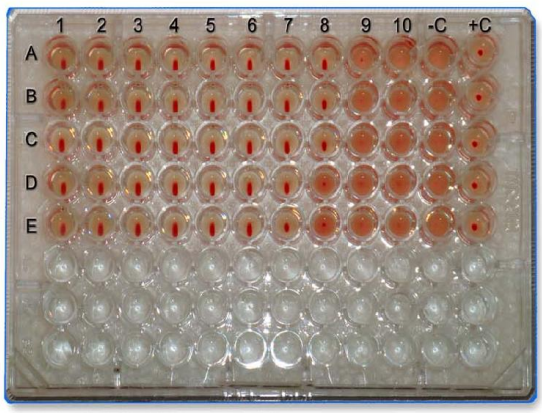

Plate 3b HI test on microtitre plate

The mean HI titre during the study period in group A, B, C, D and E were 234.66, 208.48, 212.36, 195.87 and 102.78 respectively. The mean HI titre values ranged from 85.33 to 426.66, 53.33 to 341.33, 53.33 to 341.33, 85.33 to 341.33 and 53.33 to 149.33 from 20 to 40 weeks in group A, B, C, D and E respectively. The result shows that there was a significant difference ($P<0.01$) in mean HI titre values between treatment and control groups. Among the treatment groups, group a showed highest mean HI titre of 234.66 when compared with other groups, but there was no statistically significant difference.

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