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Acquired Immune Stimulatory Effect of *Melia azadirachta* L as Feed Supplement in Growing Chicken

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Abstract: Vaccination is the only method of control of viral diseases. Layer poultry is the more species that receive more than twelve vaccines during their layer life of only 72 weeks. So utilizing the full potentials of the vaccine is a major manage mental commitment. Though many methods are available for immune stimulatory function of viral vaccines, they do have as much harm as they render benefits. Hence, the use of medicinal value of Indian herbs has been planned for a study for the purpose of using the immune stimulatory effect of the herb. Ranikhet Disease vaccine important and major viral vaccine for poultry has been selected for studying its immune response after feeding *Melia azadirachta.* Linn as feed supplement. The herbal preparation was given in the form of dry powder in two levels namely 1 and 10% for twenty days. The birds were allotted for each trial, keeping five birds as control with only normal feeding without the herb. All the birds including control were given with normal dose of live vaccine of Ranikhet disease.

Key words: Vaccination · Ranikhet Disease · *Melia azadirachta* and viral diseases

INTRODUCTION

Active acquired immunity is induced by exogenous antigens. Prevention of specific diseases in general and specific viral diseases in particular through vaccines and vaccinations is a ways better than curation after the disease. In fact, there is no curative medicine for any viral disease. Obviously, majority of the developed vaccines are for prevention of viral diseases only. Though many vaccines are quite satisfactory in preventing the expression of overt disease, the problem of breakdown of immunity has become a common attribute of any vaccination failure [1].

There are many herbal plants like *Artemisia myriantha* and *Lysimachia fortune* [2] which are known for increasing the 'innate resistance' of any biological host. In the present study *Melia azadirachta* have selected and identify immune stimulatory effect of this plant. Oral feeding of *M. azadirachta* as feed supplement, single dose of vaccination, a live egg adopted vaccine, commercial growing chickens of age sixteen weeks with normal feeding and manage mental practices, assessment of humoral immunity by micro haemagglutination inhibition assay at several periods of age of vaccination with different levels of feed supplement along body weight gain and hematological study of SGOT and SGPT enzymes were assigned as parameters for the study. The main aim of this study is to evolve a host oriented method so utilize the full potentials of an exogenous antigen namely, the Ranikhet Disease vaccine.

MATERIALS AND METHODS

Feeding of dry powdered leaves of *Melia azadirachta* Linn at a level of 1and 10% as feed supplement to 20 growing chickens of age 16 weeks constitutes the experimental groups. After 20 days, birds are inoculated with a vaccine (Newcastle vaccine- La Sota) along with 10 control birds. Birds were grown in private commercial farm and they were alive till the completion of this experiment. Body weight gain and serum antibody level for that vaccine are recorded before and after treatment and control groups by HI test and Liver function is assessed. Results are analyzed for the possible contribution of *M. azadirachta* Linn in immune response to the vaccine, in liver function and in body weight gain. The parameters were studied which contribute to immune stimulatory process were: antibody responses (HI), liver function SGOT and SGPT (J-1mole of pyruvate liberated at 37oC -l000ml serum); body weight gain and feed intake.

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Fig. 1: Schematic representation of Haemagglutination titration (HA) (virus titration) volume and end points hypothetical

	Virus titration (HA)											
Procedure	1	2	3	4	5	6	7	8	9	10	11	12
Add Saline	50	50	50	50	50	50	50	50	50	50	50	50
Add virus to First well only	50	50	50	50	50	50	50	50	50	50		
Add 1% RBC	50	50	50	50	50	50	50	50	50	50	50	50
Result											•	•
Virus dilution	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024		
											Cell control	

dicates serial dilution, : Indicates discard

Fig 2: Schematic of Heamo-agglutination inhibition (HI), serum titration assay and check titration. Volumes, dilution and end points hypothetical

		Serum Titration (HI)											
Procedure		1	2	3	4	5	6	7	8	9	10	11	12
Add Saline to all wells		25	25	25	25	25	25	25	25	25	25	25	25
Add serum to 1st to 12th		25	25	25	25	25	25	25	25	25	25	25	25
Add virus having 4HA 1st to 12th		25	25	25	25	25	25	25	25	25	25	25	25
Compensating saline		-	-	-	-	-	-	-	-	-	-	-	25
Add 1% RBC 20 all		50	50	50	50	50	50	50	50	50	50	50	50
Result		•	•	•	•	•	•	•	•				•
Check titration	Saline	50	50	50	50	50	50						
	Add virus 4 HA	50	50	50	50								
	Add 1% RBC	50	50	50	50	50	50						
	Result			•	•	•	•						
		2HA	1HA	1/2HA	1/4HA	Cell control						Serum control	
	Serum HI titre	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256				

Haemagglutination Inhibition Test

Virus Sample: Live LaSota vaccine obtained from the commercial company Indovax Pvt. Ltd. In virus titration was done [3], the last dilution of virus in which agglutination takes place is 1 HA. Division of 1HA dilution by 4 gives 4 HA dilution. The LaSota strain vaccine of RDV used in this study is a popular strain more commonly used by all farmers.

Haemagglutination Inhibition (HI) Test: The HI test was used for the titration of the serum samples collected from the birds. This test was performed in a V-bottomed microtitre plate. Fifty ~d of serial two fold dilution with normal saline of test serum, starting with 1:2 were incubated with 50~1 of 4HA units of the virus. After 30 minutes 50~d of one percent suspension of chicken erythrocytes were added. The plates were left to stand for 45 minutes at room temperature before the results were read. The plates were observed for inhibition of the haemagglutination activity of ND specific antisera. The titre was the reciprocal of the highest dilution showing complete haemagglutination inhibition.

HI End Point: In this HI test, the highest dilution of serum up to which button formation occurred is the end point and was taken as titre value.

Controls: The following controls were performed. a) Serum Control b) Cell Control c) Check Titration control.

Haemagglutination inhibition (HI) Assay Protocol HI test was followed as per Fig. 1 and 2.

Hematological Study: Aspartate amino transferase and alanine amino transferase were estimated by the standard method [4-5].

Body Weight Measurement: The body weight of each bird in 3 groups was measured at zero day, 7th day, 14th day and 21st day of post vaccination.

RESULTS

In the present study of acquired immune stimulatory effect of *M. azadirachta* in growing chicken as feed supplement, the observation in each step of the study are presented. No bird fell sick or showed clinical symptoms of any disease including the controls. The vaccine used for assessing the immune stimulatory effect is potent commercially proved vaccine for ranikhet disease of poultry, a major and important disease. In normal birds it will elicit a fairly good titre reaching a peak before 21 days.

Table 1: Heam-agglutination (Zero day, 7th day, 14th day and 21th day of post vaccination)

	HI- Titre											
	Zero day			7th day			14th day			21st day		
Sl. No	Control	Trial I	Trial II	Control	Trial I	Trial II	Control	Trial I	Trial II	Control	Trial I	Trial II
1	<16	<16	16	32	16	32	32	64	256	32	128	512
2	16	<16	16	16	16	64	64	64	512	64	128	1024
3	<16	<16	16	16	32	64	64	128	512	64	512	1024
4	<16	16	16	16	128	64	64	64	1024	64	512	1024
5	<16	16	<16	16	128	64	64	64	512	64	128	1024
6	<16	<16	16	16	128	64	64	128	1024	64	128	1024
7	<16	<16	<16	128	128	64	32	128	128	64	256	512
8	<16	<16	16	128	128	64	64	128	128	64	256	512
9	16	<16	16	256	128	64	64	256	128	64	256	512
10	<16	16	16	258	128	64	64	256	512	64	256	1024
Mean value	4.8	4.8	3.2	12.8	75.2	96	57.6	128	473.6	57.6	256	819.2

Table 2: Comparison of SGOT and SGPT levels (U/L-µmole of pyruvate liberated at 37oC/min/1000 ml serum) of Control, Trial I and II on 21st day of post vaccination

	SGOT (U/L)			SGPT (U/L)		
Sl. No	Control	Trial I	Trial II	Control	Trial I	Trial II
1	12.1	8	8	13.4	8	8
2	13.5	8.2	8	15.6	8	8.2
3	12.3	8.5	8.2	13.8	8.5	8
4	14.3	8	8	11.8	8	8.2
5	11.7	8	8.2	11.1	8.2	8
6	12.3	8.5	8.2	12.4	8.2	8.2
7	14.2	8.2	8	15.2	8.2	8
8	13.9	8	8.5	14.9	8	8.2
9	13.6	8	8	12.6	8	8
10	15.4	8	8	11.4	8	8
Mean value	13.3	8.14	8.11	13.2	8.11	8.08

Table 3: Comparison of average body weight gain (in grams) of birds in all the groups and % weight gain

		Average body wt in g/bird					
Sl. No	Group	Zero day	7th day	14th day	21st day	Average body wt gain / bird in g	Average body wt gain / bird in %
1	Control	574	616	673	752	178	31.0
2	Trial I	594	657	728	847	253	42.6
3	Trial II	552	670	810	933	381	69.0

Table 4: Comparison of feed intake by control, Trial I and Trial II birds during the study

Sl. No	Group	Total feed intake of all birds in 41 days in kg	Feed intake per birds in 41 days in kg	Feed intake per birds / days in g
1	Control	26	2.6	63.4
2	Trial I	24	2.4	58.5
3	Trial II	20	2.0	48.8

Before inoculation the vaccine was checked for its in vitro haemagglutination activity which was found to be 1024 (Table 1).

The mean HI titre estimated just prior to vaccination (Zero day) and on 7th day, 14th day and 21st day of post vaccination of control were 4.8, 12.8, 57.6 and 57.6, respectively

The mean HI titre estimated just prior to vaccination zero day, 7th day, 14th day and 21st day after vaccination of control were 4.8, 12.8, 57.6 and 57.6, respectively. The mean HI of trial I and trail II were (4.8, 75.2, 128 and 256) and (3.2, 96, 473.6 and 819.2), respectively (Table 2).

The average SGOT and SGPT levels of control, trail I and trail II were (13.3 U/L and 13.2' U/L), (8.14 and 8.11) and (8.11 and 8.08), respectively (Table 2).The average body weight on zero day of control, trial I and trial 11 were 574, 594 and 552 grams ,respectively. The average body weight values on ill, 14th and 21st day were (616, 657 and 670), (673, 728 and 810) and (752, 847 and 933), grams respectively. The average body weight gain of control, trial I and trial II were 178,253 and 381 grams, respectively. The percent body weight gains were 31, 42.6 and 69 respectively (Table 3). Total feed intake by control, trial I and trial II were 26, 24 and 20 kilogram, respectively. Feed intake during the study by control, trial I and trial II were 2.6, 2.4 and 2.0 kilogram respectively. Feed intake per bird per day was 63.4, 58.5 and 48.8 gram, respectively (Table 4).

DISCUSSION

The egg poultry production life is about 72 weeks; they receive more than twelve vaccines and about twelve vaccinations. Both vaccines and vaccinations are induced stress factors to the birds and are evils, because viral diseases can not be cured and vaccination is the only way out for prevention. Vaccination failures and break down of immunity are the common incidences in poultry health management, however potent the vaccines may be. So utilizing the full potentials of the vaccines to get maximum immunogenic benefit is an important aspect to the borne in mind.

Though there are many exogenously added immunomodulants, collectively called adjuvants, like vaccines, they may also fail because the reasons for breakdown of immunity beyond the purview of vaccines are also common to these immunomodulants. Or in other words, the reasons for each breakdown of immunity or vaccination failures reside in the host namely the bird. Increasing awareness on herbs and plants in general and medicinal herbs in particular, was the root cause for designing a study to prepare and modulate the host towards an exogenously given vaccine with an anticipation of eliciting a maximum seroconversion of the vaccine.

Alla *et al* [3] have established that an HI titre of more than 2 (96) was required for the bird to get protected from death, the maximum severity of the disease and a titre of more than 26.5 (96) was required for the bird to be free from egg drop due to Ranikhet disease, the minimum and the earliest disease manifestation [6-8].

In the present study, the trial I and trial II excel the control as per standards (Table I) [3]. Though trial II yielded an exorbitantly high HI titre compared to trial I, by cost wise trial I is one tenth of the cost of trial II. But trial I in no way inferior in terms of HI titre, as it elicited one and half log (base 2) more titre than [3] standards to prevent even the minimum severity of the disease. A 256 serum titre for Ranikhet disease in considered a good solid protection. Hence trial I with 1% level of *M. azadirachta* added as feed supplement qualified for selection between 1% and 10%.

Toxicity is the major and more common attribute causing break. Mycotoxins are the common contaminants of poultry feed because of groundnut cakes, fish meal, bone meal and maize [4]. “Fumonisin", a group of mycotoxins produced by *Fusarium mouiliforme* frequently affecting Indian maize, significantly toxic even at sub-lethal dose [9]. Not necessarily toxins even antibiotics in excess doses as residual contaminants may cause toxicity. Liver function in the form of SGOT and SGPT levels can be a measure of sub lethal toxicity because liver the only detoxifying organ and reported that a drastic reduction in SGOT (AST) and SGPT (ALT) indicated a sound liver function without sub lethal dietary toxicity [10].

In the present study, the reduction in the level of trial I and trial II compared to controls is an indication of a good condition of the liver function and this might be one of the reasons that there is a good immune response in trial I and trial II. Though the controls showed no phenotypic lethality a high SGOT and SGPT revealed sub-lethal toxicity either dietary or water (Table II). The quantitative feed restriction and limited time feeding contributed better feed intake, better egg production and efficient feed conversion, Body weight gain and feed intake may give the feed conversion efficiency, another indication of health status [11]. In the' present study, trial I and trial II are better than control and when compared to trial I, the trial II is greater on the basis of body weight gain (Table III). In the present study, feed intakes by control were greater than Trial I and Trial II. When trial I and II are compared, feed intake by trial II is less (Table IV). Trial II is double the efficiency of trial I in feed conversion. Body weight, feed intake and the corresponding feed conversion efficiency recorded also reflect the liver function level corresponding to the group. More feed intake and less feed conversion is one of the basic informs on stress factor and a stress on liver.

The trial conducted in this study had only one variable namely the percent addition of the herb and the period of feeding as constantly 41 days namely 20 days before vaccination and 21 days after vaccination. However, the age of the birds selected was 14th to 20th week-the crucial pre-lay period involving more stress. So for all practical purposes 5 to 6 weeks before start of lay can be selected for feeding the herb and hence results of this study can be completely applied.

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