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Effect of Neem, Turmeric, Vitamin E and Their Combinations on Immune Response in Broilers

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Abstract: An experiment was conducted to study the efficiency of inclusion of neem, turmeric, vitamin E and its combinations on humoral immune response against newcastle virus and cell mediated immune response [Dinitrochlorobenze (DNCB) test – neck and foot web] for a period of six weeks with 288 day old Raja II broiler chicks. Basal diet was supplemented with *Azadirachta indica* (Neem @ 8g/kg feed), *Curcuma longa* (Turmeric @ 2g/kg feed) and Vitamin E (@ 0.2g/kg feed) individually and in combination to form eight test diets. Each test diet was fed ad libitum for 42 days. Results of Heamagglutination inhibition (HI) titer revealed a significant difference ($P<0.05$) among various treatment groups at 3rd week of age and remained non-significant at first and fifth week of age. Statistical analysis revealed a significant difference ($P \leq 0.05$) in mean HI values of group fed with neem and its combination of turmeric (T_5), vitamin E (T_7) and combination of both turmeric and vitamin E (T_8) when compared to control. However use of turmeric, vitamin E and its combination showed no significant effect on mean HI titer values, although numerically a positive impact was observed. DNCB test revealed that inclusion of neem, turmeric and Vitamin E significantly improved the cell mediated immune response in broilers either alone or in combination. However, combinations containing turmeric and vitamin E had better results compared to neem fed and control diet fed broilers.

Key words: *Curcuma longa* · DNCB · Humoral · Cell Mediated Immunity · Broilers

INTRODUCTION

Poultry sector in India has developed unprecedented growth during last three decades and now transformed itself into the status of an industry. Today, India ranks third largest producer in eggs [1] and fifth largest producer in broiler chicken in the world [2]. The poultry production as practiced today is a specialized one and concentrating more on the use of high performance birds. The major factors for successful poultry production are high genetic potential, balanced nutrition and health maintenance. On the other hand, there is a major demand to produce high quality poultry meat and egg at low price without rely on antibiotics and other medicines in poultry feed and water.

Many synthetic drugs and growth promoters are supplemented to the broilers to effect rapid growth, but their use have shown many disadvantages like high cost, adverse side effect on health of birds and long residual properties etc. So, scientists are again concentrating on the use of our ancient medicinal system to find beneficial herbs and plants, which can be safely used to increase the production.

Neem (*Azadirachta indica*) and turmeric (*Curcuma longa*) are herbal plants of India which have demonstrated to to exhibit various beneficial pharmacological properties including immunomodulatory effect [3, 4] in broilers. In addition, Vitamin E along with selenium has been found to be essential for the proper development of the immune system [5] and for the overall

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immunocompetence of growing broilers. However, use of these medicinal plants or feed additives in diets of broiler chicken with the objectives of improving their health and immune status has not been fully addressed. Hence, the present study has been proposed to evaluate the effeciecny of neem, turmeric, vitamin E and their combinations in the immune response in broilers.

MATERIALS AND METHODS

Experimental Birds: Two hundred and eighty eight indigenously developed Raja II colored broilers were procured from the Department of Poultry Science, Veterinary College, Bangalore. The birds were divided among 8 treatments with each treatment having 3 replicates with 12 birds in each replicate. Twenty four pens were made and the replicates were randomly allotted using lottery method under deep litter system and reared for 6 weeks.

Diets: Diets were formulated using maize and soybean meals. The broiler starter and finisher rations were formulated as per BIS (1992). Starter mash was fed from one to 21 days and finisher from 22 to 42 days. A practical type of broiler diet was compounded to serve itself as control (T1). Basal diet was fortified with *Azadirachta indica* (Neem) powder @ of 8 gm/kg feed (T2), *Curcuma longa* (turmeric) @ of 2 gm/kg feed (T3) and Vitamin-E @ of 0.2 gm/kg feed (T4) and likewise 4 experimental diets were prepared using their combination and were designated as T5, T6, T7 and T8 (Table 1).

Assessment of Humoral Immune Response: Heamagglutination inhibition test was used to check humoral immune response. The micro-test method described by Allan and Gouch, [6] was used for detection of HI titers from serum samples collected on 7th day before immunization, 3rd wk and 5th wk of post immunization of birds to assess the titers and their relation with the test feed. The HI test was done manually by â- procedure in 'U' bottom micro-plates using diluters, droppers and 4 Haemagglutination (HA) units of Newcastle Disease viral antigen. The titers were expressed as the reciprocal of highest dilution of serum showing the heamagglutination inhibition or button formation.

Assessment of Cell Mediated Immune Response (CMI): Assay of CMI using 2, 4-Dinitrochlorobenze (DNCB). Cutaneous hypersensitivity test was performed as per Hari Babu *et al*. [12]. At the end of the experiment i.e., 42nd day DNCB test was performed. Six birds from each treatment were taken. Three birds were sensitized in the foot web region and three were sensitized in the neck region with 0.05 ml of 2% DNCB in acetone using hypodermal syringe. The thickness of the skin and foot web before challenge (0 hrs) and at 24 and 48 hrs was recorded using vernier calipers.

Table 1: Description of Experimental Treatments

	Experimental diets		
Treatment	Neem (%)	Turmeric (%)	Vitamin-E (%)
T1(control)	-	-	-
T2	0.8	-	-
T3	-	0.2	-
T4	-	-	0.02
T5	0.8	0.2	-
T6	-	0.2	0.02
T7	0.8	-	0.02
T8	0.8	0.2	0.02

Data pertaining to various parameters obtained during the experiment was analyzed as Completely Randomized Block Design according to the methods described by Snedecor and Cochran [7].

RESULTS

Humoral Immunity: The results of the effect of feeding neem, turmeric, vitamin E individually and in combination on Heamagglutination inhibition titer against newcastle disease vaccine at 1st, 3rd and 5th week of age are presented in Table 2.

The analysis of variance revealed a significant difference ($P<0.05$) among various treatment groups at 3rd week of age and remained non-significant at first and fifth week of age. Statistical analysis revealed a significant difference ($P\leq0.05$) in mean HI values of group fed with neem and its combination of turmeric (T_5), vitamin E (T_7) and combination of both turmeric and vitamin E (T_8) when compared to control. However use of turmeric, vitamin E and its combination showed no significant effect on mean HI titer values, although numerically a positive impact was observed.

Cell Mediated Immune Response: The results of feeding neem, turmeric, vitamin E individually and in combination on cell mediated immune response to DNCB at twenty four and fourty eight hours at foot web and neck regions are presented in Tables 3 and 4. Analysis of variance revealed significant difference ($P\leq0.05$) among various treatments on cell mediated immune response measured at twenty four and fourty eight hours of post inoculation.

Table 2: Effect of neem, turmeric, vitamin E(VE) and their combination on HI titer against NDV (mean± SE) in broilers

		HI titer		
	Treatments	1st wk NS	3rd wk	5th wk NS
T_1	Basal diet	16±00	048±16^{a}	064±00
T_2	Neem (0.8%)	16±00	192±64^{ab}	128±00
T_3	Turmeric (0.2%)	32±00	128±00^{ab}	096±32
T_4	Vitamin E (0.02%)	32±00	128±00^{ab}	096±32
T_5	Neem+ Turmeric	24±08	256±00^{a}	192±64
T_6	Turmeric + VE	32±00	192±60^{b}	096±32
T_7	VE + Neem	24±08	256±00^{a}	128±00
T_8	Neem +Turmeric+ VE	24±08	256±00^{a}	128±00

NS- Non- significant. Means bearing at least one common superscript column wise does not differ significantly ($P \leq 0.05$)

Table 3: Effect of neem, turmeric, vitamin E and their combination on cell mediated immune response (mean± SE) in broilers (foot web)

		Thickness (mm)		
	Treatment	0 hr NS	24 hr	48 hr
T_1	Basal diet	0.88±0.13	1.35±0.06^{ae}	2.50±0.26^{ae}
T_2	Neem (0.8%)	0.97±0.04	2.75±0.06^{ab}	3.74±0.06^{ab}
T_3	Turmeric (0.2%)	0.85±0.12	5.15±1.05^{bcf}	5.95±1.15^{bcf}
T_4	Vitamin E (0.02%)	0.89±0.06	2.57±0.05^{ca}	6.61±0.19^{dc}
T_5	Neem+ Turmeric	1.09±0.18	2.74±0.09^{ba}	3.63±0.19^{ef}
T_6	Turmeric + VE	0.97±0.03	2.95±0.15^{abc}	3.53±0.07^{fe}
T_7	VE + Neem	0.72±0.12	2.24±0.06^{cab}	5.10±0.46^{bce}
T_8	Neem +Turmeric+ VE	0.81±0.04	3.36±0.21^{ef}	4.05±0.14^{aef}

NS-Non-significant. Means bearing atleast one common superscript column wise does not differ significantly ($P<0.05$)

Table 4: Effect of neem, turmeric, vitamin E and their combination on cell mediated immunity (mean± SE) in broilers (neck region)

		Thickness (mm)		
	Treatment	0 hr NS	24 hr	48 hr
T1	Basal diet	0.9±0.11	1.07±0.12^{a}	1.35±0.19^{ae}
T2	Neem (0.8%)	0.78±0.01	2.00±0.10^{b}	3.07±0.39^{abf}
T3	Turmeric (0.2%)	0.84±0.06	2.10±0.10^{b}	3.45±0.25^{bcg}
T4	Vitamin E (0.02%)	1.02±0.14	2.21±0.22^{b}	3.03±0.06^{ad}
T5	Neem+ Turmeric	0.84±0.07	2.03±0.07^{b}	3.03±0.38^{abc}
T6	Turmeric + VE	0.92±0.05	1.85±0.05^{b}	2.54±0.23^{cab}
T7	VE + Neem	1.06±0.02	1.77±0.04^{b}	2.74±0.16^{bca}
T8	Neem +Turmeric+ VE	0.88±0.13	1.94±0.10^{b}	4.91±0.65^{efg}

NS* Non-significant. Means bearing atleast one common superscript column wise does not differ significantly ($P<0.05$)

DNCB at Foot Web: Among various herbal supplemented groups, the turmeric (5.15 mm) showed significantly ($P \leq 0.05$) higher cell mediated immune response when compared to control group (1.35 mm) and remained non-significant with neem and vitamin E alone fed groups. There was also significant difference ($P \leq 0.05$) in immune response at foot web at twenty four hour among treatment groups, T_2 (Neem), T_4 (Vitamin E), T_5 (Neem+ Turmeric), T_6 (Turmeric+ Vitamin E), T_7 (Neem+ Vitamin E) and T_8 (Neem+ Turmeric + Vitamin E).

Among various T2, T3 and T4, birds fed on diet T4 recorded significantly ($P \leq 0.05$) higher cell mediated immune response to DNCB when compared to T1 and T2 and remained non-significant with T3. Addition of neem to turmeric containing diet showed a significantly ($P \leq 0.05$) higher immune response as compared to its positive control (Neem alone), whereas addition of turmeric and vitamin E to neem and turmeric containing diets showed a non-significant increased in thickness of skin as compared to their respective control [10].

DNCB at Neck Region: Supplementation of either neem, turmeric, vitamin E individually and in combination had no significant effect on foot web thickness when compared to T_1 (control). The highest response to DNCB at neck region at 24 hr among treatment groups was measured in vitamin E group (2.51 mm) as against lowest was observed in control diet (1.07 mm). Addition of neem, turmeric, vitamin E individually or its combination to control diets significantly ($P<0.05$) increased immune response to DNCB at neck as compared to control group and remained non-significant among each other. Addition of neem alone and its combination with turmeric and vitamin E had no significant difference in immune response when compared with control, but neem+ turmeric+ vitamin E group measured significantly ($P \leq 0.05$) higher thickness of neck as compared to other groups and control.

DISCUSSION

Humoral Immunity: The results of the present study was similar to Sadekar *et al*., [8] who reported that feeding of powdered neem leaves significantly enhanced antibody titers against Newcastle disease virus (NDV) antigen. The HI titer values at fifth week revealed a non significant difference between the treatment groups and a decreasing trend in the titer values in all the treatments compared to values of third week. This might be due to the fact that the booster dose of vaccine was not provided in fourth week of study. Contrary to the results observed in the study in terms of vitamin E and turmeric Jagadeeswaran [9], observed a significantly higher antibody response in birds supplemented with turmeric and vitamin E against NDV.

DNCB Test: This findings of the present study was consistent with Hesabi Nameghi *et al*. [11] who reported significantly higher cutaneous basophil hypersensitivity response on inclusion of 50 and 75 IU of vitamin E per kg broiler diet. This increase in cell-mediated immune response could be due to anti-oxidant properties of curcumin and vitamin E which protects immunological tissue from destruction [12]. A similar observation of higher immune response due to dietary inclusion of turmeric was recorded by Mehala and Moorthy [13] in broiler chicks.

CONCLUSION

Inclusion of neem, turmeric, Vitamin E and their combinations in diet of broilers have been found to be effective in improving both humoral immunity against NDV and also cell mediated immunity in broilers compared to that of control diet. However, further studies are essential to assess the impact of these additives on performance interms of body weight gain and feed conversion efficiency. In addition the economics involved in the inclusion of these natural additives in diet of broilers needs consideration before final conclusion is drawn.

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