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Immunomodulatory Effect of Tulsi (*Ocimum Sanctum*) Leaves Powder Supplemented in Broilers

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Abstract: ***An experiment was conducted to determine the immunomodulator of broilers fed diets supplemented with a Tulsi (Ocimum sanctum) leaf powder. A total of 72 (Arbor-Acres) day old chicks were used in this study. Four levels of a Tulsi (Ocimum sanctum) leaf powder at the rate of 0.00%, 0.25%, 0.50%, and 1% were incorporated into the basal diet for six weeks. Feeding period for all groups was lasted for 42 days. Birds were vaccinated with ND virus and serum was assessed for humoral immune response (HI). Cell mediated immune response (CMI) was assessed as increase in IDF thickness after and before PHA-P injection. Supplementation of Tulsi at either dose showed improved HI and CMI responses. Increasing the supplementation level of herbal Tulsi (from 0.25 to 1%) improvement HI and MI. The humoral immune response (HI) and Cell mediated immune response (CMI) significantly higher in 1% Tulsi leaf powder group (T_4) as compared control (T_1) in a column (4^{th} week & 6^{th} week). The study indicated that Tulsi at 1% could be used as natural supplement to improve immune response in broiler chicken.***

Keywords: Broilers, Humoral immune, cell mediated immune, immunity and Tulsi

1. Introduction

Intensive poultry production with fast growing strains and high stocking densities are usually susceptible to infectious agents due to varied reasons and one such important reason is reduced immune potential. The impact analysis of this practice reveals many negative effects namely failure of treatment, escalation of treatment cost and mortality in flocks some times. Many medicinal plants showing immunomodulatory activity have been used instead of drugs because of their low toxicity for the host system, adequate absorption and capability to reach the target organ without much degradation by host enzymes. *Ocimum sanctum* Linn. (Lamiacea) commonly known as holy basil in English, Tulsi in Hindi and Tamil is an Indian medicinal plant which is known to have ethno-medical uses such as hepatoprotective, antihyperlipidaemic, myocardial salvaging and immunostimulant effect in man and animals. Hence, the present study was carried out to explore the possible immunomodulatory effect of *Ocimum sanctum*in broilers. Recently, there is an increasing search for potential drugs especially of plant origin that are capable of modifying immune responses with comparatively less side effects. Tulsi (*Ocimum sanctum*) is a popularly known traditional herb and possess numerous medicinal values. Studies revealed that Tulsi had immuno modulatory activity (Mediratta et al., 1988). However information pertaining to efficacy of Tulsi in amelioration of heat stress induced changes in immunity and cortisol level is sparse. Hence, the present study was aimed at evaluating the immuno- modulatory and cortisol sparing effect of Tulsi in heat stressed broilers.

2. Material and Method

Seventy two day old of same hatch were randomly distributed into four groups i.e. T_1 (Control), treatment T_2, T_3 and T_4 with six sub groups comprising of three birds in each. Broilers in T_1 were fed diet as per (NRC, 1994) standard (CP 22 and ME 2900) but broilers in T_2, T_3 and T_4 were fed standard ration supplemented with Tulsi (O*cimum sanctum)* leaf powder at the rate of 0.00%, 0.25%, 0.50%, and 1% were incorporated into the basal diet. All broilers were offered feed and water *ad libitum* throughout the experimental period. They were housed in metal type battery cages in small animal laboratory of S.S. and AH Dairying, SHIATS Allahabad. A bulb of 15 watt was left on in each cage. Initial weight of each chick was recorded on arrival and then weekly.

Table 1: Ingredient and nutrient composition of experimental diet (%DM)

Ingredients (%)	*Broiler starter (0 – 21 days)*	*Broiler finisher (22 – 42 days)*
Maize	60.00	63.00
Ground nut cake	23.11	18.00
Fish meal	12.60	14.60
Premix*	2.50	2.50
Salt	0.30	0.30
Methionine	0.10	0.01
Lysine	0.10	0.01
Di-calcium phosphate	1.20	1.20
Total	100	100
Calculated Chemical analysis		
Moisture (%)	6.29	6.22
Crude Protein (%)	23.29	21.28
Total Ash (%)	8.02	9.34
CP	22.00	19.00
ME (Kcal/Kg)	2900	3000
Calcium (%)	0.69	0.52
Available phosphate (%)	0.74	0.69
Methionine(%)	0.33	0.31
Lysine (%)	1.19	1.08

*Premix (2.5%) Provided the following (Per Kg of complete diets). Vit A. 367500 IU, 133500 IU Vit. D3, 1920 mg Vit.E, 84.42 Vit. K3, 50 mg Vit. B1, 150 mg Vit. B2, 500 mg Vit. B3, 177.5 mg Vit. B6, 0.8 mg Vit. B12, 600 mg Vit. PP, 24.5 mg folic acid, 27 mg biotin, 5767.5 mg choline, 2667 mg Fe, 333.75 mg Cu, 3334.06 mg Mn , 203 mg Co , 2334.38 mg Zn, 100.75 mg Ca , 10 mg Se, 65446.46 mg Ph, 36667.5 mg DLMithionine, 200.02mg, Ethoxyquin,50mg Flavophospholipol, 30g Fish meal, 1800g wheat bran.

Green Tulsi (O*cimum sanctum)* leaves were dried for three to four days initially and then in oven at 60ºC up to moisture content level below 10%. Then the leaves were crushed manually to make it fine. It was passed through fine meshed wire sieve to obtain uniform powder. Then it was mixed with standard feed mixture according to the ratio mentioned. Chicks were provided 0.8 sq.ft/bird space. Cages, feeders, waterers, and other equipments were properly cleaned disinfected and sterilized before use. The waterers were disinfected with 0.02% KMnO4 solution every day. Birds were vaccinated with Lasota strain of ND on 7th day of age by intraocular route followed by a booster using R2B strain on 21st day age. At the end of 4th and 6th wk of age sera samples were collected from twelve birds from each group to assess immune response. The humoral immune response was assessed against ND virus by haemagglutination inhibition (HI) test (Cunningham, 1966). Cell mediated immune response (CMI) was assessed by injecting 0.1 ml of reconstituted Phyto haemogglutinin-p in PBS (Phosphate buffer saline) intradermally in the right 3-4 inter digital fold (IDF) of birds at 4th and 6th wk of age. The CMI response was calculated as increase in IDF thickness after and before antigen injection. The data was subjected to statistical analysis by applying two ways ANOVA using statistical package SPSS (10.0 versions). Difference between means was compared using Duncan's multiple comparison tests (Duncan, 1955).

3. Results and Discussion

In the present study table 1 humoral immune response assessed as $\log^2$ titer against ND vaccine was significantly higher in 1% Tulsi leaf powder group (T_4) as compared control (T_1) in a column (4^{th} week) but the $\log^2$ titer non significantly higher in 1% Tulsi leaf powder group (T_4) as compared control (T_1) in a column (6^{th} week). In table 2 the cell mediated immune response by PHA – P inoculation was affected by increasing significantly skin thickness 1% Tulsi leaf powder group (T_4) as compared control (T_1) in a column (4^{th} week) but the $\log^2$ titer non significantly higher in 1% Tulsi leaf powder group (T_4) as compared control (T_1) in a column (6^{th} week). The inclusion of tulsi at either concentration showed improved HI titer and CMI response (Table 1 and 2) hence Increasing the supplementation level of tulsi increasing the immunity status. Herbs can influence selectively the microorganism by an antimicrobial activity thus favors better nutrient utilization and absorption or the stimulation of the immune system (as per Wenke, 2003). In the present study, supplementation diet with Tulsi improved immune responses which might be due to immunostimulatory effect of eugenol and other essential oils present in Tulsi (Sen, 1993). The present results are in accordance with report by mediratta et al. (1988) in rat fed diets supplemented with Tulsi. However, the improvement observed in immune response with 1% Tulsi supplementation.

Table 1: Humoral immune response in broilers supplemented with Tulsi (Ocimum Sanctum) leaf powder

Treatment	*H.I. (log^2 titer)*	
	4^{th} week	*6^{th} week*
T_1 (control)	7.37 ± 1.32^{a}	8.23±1.06
T_2 + Tulsi (0.25%)	8.02 ± 1.04^{ab}	9.32±1.31
T_3 + Tulsi (0.5%)	8.68 ± 1.21^{ab}	9.72±1.24
T_4 + Tulsi (1%)	9.72 ± 1.28^{b}	10.03±1.03

Means with different superscripts in a column differ significantly at P<0.01

Table 2: Cell mediated immune response in broilers supplemented with Tulsi (Ocimum Sanctum) leaf powder

Treatment	Increase in skin thickness (mm) against PHA-Pm—	
	4^{th} week	6^{th} week
T_1 (control)	0.43 ± 0.21^{a}	2.06±0.96
T_2 + Tulsi (0.25%)	0.73 ± 0.34^{ab}	2.65±0.81
T_3 + Tulsi (0.5%)	1.26 ± 0.61^{ab}	2.69±0.74
T_4 + Tulsi (1%)	2.16 ± 0.68^{b}	2.72±0.93

Means with different superscripts in a column differ significantly at P<0.01

The present study resulted that Supplementation of Tulsi at 1% the significantly effect the stimulated the immune response. But considering the safety and easy availability of this herb, Tulsi supplementation may be considered as natural alternative in improving the immune response.

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