IMMUNOMODULATORY AND CORTISOL SPARING EFFECT OF TULSI (*OCIMUM SANCTUM*) IN HEAT STRESSED BROILERS

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ABSTRACT

An experiment was conducted in hot summer months with 120 broiler chicks in 5 groups which were given basal diet(BD), BD+vitaminE(200mg/Kg), BD+vitaminE (200mg/kg)+ selenium (0.15mg/kg),BD+Tulsi(0.25%) and BD+Tulsi(0.5%), respectively. A control group of 24 birds was raised separately in stress free environment. Birds were vaccinated with ND virus and serum was assessed for humoral immune response (HI) and cortisol levels. 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 but levels were lower than that obtained with vitamin E alone or in combination with selenium. Increasing the supplementation level of herbal Tulsi (from 0.25 to 0.5%) did not contribute to any additional improvement. The study indicated that Tulsi at 0.25% could be used as natural supplement to improve immune response and lower cortisol level during heat stress condition in broiler chicken.

Key words: Broilers, Cortisol, Heat stress, Immunity and Tulsi

INTRODUCTION

Heat stress is one of the most important stressor especially in hot regions of the world. Adaptation to heat stress requires physiological integration of many systems such as endocrine, cardio respiratory and immune systems. Hence, heat stress induces hormonal changes such as increased corticoid secretion (Brown and Nedtor, 1973) at temperatures above (or) below thermo neutral zone. Heat stress increases plasma corticosterone levels in cockerels (Ben Nathan *et al.,* 1976) and in chicken (Mahmoud *et al*., 2004). The intensity and duration of stress induced by environmental temperature may impair the immune system of poultry (Donker et al., 1990). 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) and normalizing effect on increased corticosterone level in noise

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stress (Shembulingam et al., 1997) in rats. 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.

MATERIALS AND METHODS

One hundred and twenty day old vencobb broiler chicks were randomly divided into 5 groups maintained on standard managemental practices at Poultry Experimental Station, Rajendranagar, Hyderabad for 42 days in hot summer months (temperature 37°C to 45°C). The dietary groups were Vuiz.Group I (G-I) fed standard maize soya bean meal diet (BD) (ICAR 1998). Group II (G-II) was given BD supplemented with 200 mg/kg vitamin E (Qualigens Pvt Ltd India). Group III (G-III) was fed with BD with vitamin E (200mg/kg) + 0.15 mg/kg Selenium (SelplexTM Altech). Group IV and V were supplemented with Tulsi powder (M/S I Dreamz Health care, Banglore, India) at 0.25 and 0.5% level of basal diet, respectively. Additionally a control group of 24 birds was maintained in heat stress free environment which acted as stress free control. 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 and cortisol hormone.

Cortisol hormone concentration was estimated in serum using UBI MAGIWEL cortisol quantitative SH101 in a solid phase enzyme linked immunosorbent assay (ELISA) kit (Travis, 1976). 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 way ANOVA using statistical package SPSS (10.0 version). Difference between means was compared using Duncan's multiple comparison tests (Duncan, 1955).

RESULTS AND DISCUSSION

In the present study, untreated birds group exposed to heat stress (HS) showed elevated levels of cortisol (Table 1) compared to heat stress free (control) group which could be due to release of corticosteroids by adrenal gland to increase the supply of energy to HS birds to cope up with the stress. Birds supplemented with vitamin E had significantly ($P \leq 0.01$) reduced cortisol concentration and the reduction was more significant when Se was supplemented to vitamin E. Both vitamin E and Se prevents oxidative stress in broilers (Roch et al., 2000) and hence synergistic effect of both these micronutrients was observed in the

present study. Inclusion of Tulsi powder at either dose could cause a significant ($P \leq 0.01$) reduction in cortisol concentration at both 4th and 6th wk of age compared to HS group indicating the cortisol sparing effect of this herb during stress. A similar reduction in cortisol was reported earlier with Tulsi in rats subjected to physical stress due to swimming (Bhargava and Singh, 1981). Increasing in the supplementation level of Tulsi from 0.25 to 0.5% had not contributed to any additional advantage in lowering cortisol concentration. However, the reduction in cortisol concentration observed with Tulsi supplementation at either dose was lower than that obtained with Vitamin E as alone or in combination with selenium.

Humoral immune (HI) response assessed as $\log^2$titers against ND vaccine was significantly ($P \leq 0.01$) higher (7.77 at 4th wk and 8.03 at 6th wk) in stress free group in comparison to HS group (3.06 at 4th wk and 2.91 at 6th wk respectively). The cell mediated immune (CMI) responses by PHA– P inoculation was affected by heat stress as indicated by increase in skin thickness which was least (0.09 mm at 4th wk and 0.26 mm at 6th wk) in heat stressed birds compared to the values obtained with stress free birds (0.43 at 4th wk and 2.06 at 6th wk). The decrease in immunity in HS group might be due to high cortisol from adrenal as observed in the present study due to its immunosuppressive effect (Golub and Gershwin, 1985). Vitamin E has been reported to protect cells involved in immune response such as lymphocytes, macrophages and plasma cells against oxidative damage (Meydani and Blumberg, 1993) which could be attributed for higher immune response with vitamin E supplementation in present study. Addition of vitamin E to the diet significantly ($P<0.01$) improved the HI titers (3.41 at 4th wk and 5.82 at 6thwk) and CMI response (0.32 and 1.47 mm at 4th and 6th wk respectively). Furthur significant ($P<0.01$) improvement was obtained with the addition of Se to vitamin E in diets with an increase in HI titers to 4.92 and 7.01 and CMI response to 0.39 and 1.82 mm at 4th and 6th wk, respectively compared to the values of HS birds (Table 2 and 3).

Inclusion of Tulsi at either of concentration showed improved HI titers and CMI response (Table 2 and 3). Increasing the supplementation level of Tulsi from 0.25% to that of 0.5% did not contribute to any additional improvement in immunity status. Herbs can influence selectively the microorganisms by an antimicrobial activity thus favour better nutrient utilization and absorption or the stimulation of the immune system (Casper Wenk, 2003). In the present study, supplementing 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 rats fed diets supplemented with Tulsi. However, the improvement observed in immune response with Tulsi supplementation was lower than that observed with vitamin E as sole supplement or in combination with Selenium. Whether the

dose employed in the present study is not sufficient or the form in which Tulsi was supplemented could not yield its active principle to the maximum extent needs further investigation.

The present study revealed that heat stress had resulted in increased cortisol concentration and reduced immune activity in broilers. Supplementation of Tulsi at 0.25% reduced the serum cortisol concentration and stimulated the immune system. However, the cortisol lowering and immunomodulatory effect of Tulsi was lower than that obtained with supplementation of synthetic antioxidant minerals (vitamin E alone or incombination with selenium). But considering the safety and easy availability of this herb, Tulsi supplementation may be considered as natural alternative in improving heat stress induced hormonal and immune changes.

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Table 1.
Serum cortisol concentration (µg/dl) in heat stressed broilers supplemented with antioxidants.

Treatment	Cortisol		
	-4th wk	**6th wk**	**Average+SEM**
Stress free control	0.36±0.021 ^{j}	1.95±0.031 ^{f}	1.15 ± 0.172
Heat stressed (HS)	4.54 ± 0.021^{b}	8.77 ± 0.022^{a}	6.64 ±0.181
HS+ Vitamin E (200 mg/kg)	1.04 ± 0.033^{h}	2.72 ±0.023^{d}	1.88 ± 0.179
HS+ Vitamin E (200 mg/Kg)+Se (0.15mg/kg)	0.97 ± 0.032^{i}	2.23 ± 0.024^{e}	1.59 ± 0.171
HS+Tulsi (0.25%)	1.33 ± 0.024^{g}	3.65 ± 0.021^{c}	2.49 ± 0.169
HS+Tulsi (0.5%)	1.30± 0.021^{g}	3.53 ± 0.032^{c}	2.41 ± 0.172
P value	0.001	0.001	

Diet Period interaction- P≤0.001
Means with different superscripts in a row and column differ significantly at P≤0.01

Table 2.
Humoral immune response in heat stressed broilers supplemented with antioxidants.

Treatment	H.I. (log^2titers)		
	4th wk	6th wk	Average+SEM
Stress free control	7.77 ±0.031 [b]	8.03± 0.071 [a]	7.91 ±0.032
Heat stressed (HS)	3.06± 0.062 [h]	2.91 ± 0.041 [i]	2.98 ± 0.031
HS+ Vitamin E (200 mg/kg)	3.41 ± 0.042 [f]	5.82 ±0.046 [c]	4.58 ± 0.033
HS+ Vitamin E (200 mg/kg)+Se (0.15mg/kg)	4.92 ± 0.073 [e]	7.01± 0.035 [b]	5.96 ±0.027
HS+Tulsi (0.25%)	3.32 ± 0.054 [g]	5.28 ± 0.061 [d]	4.32± 0.042
HS+Tulsi (0.5%)	3.33 ±0.055 [g]	5.32 ±0.033 [d]	4.32 ± 0.029
P value	0.001	0.001	

Diet period interaction P≤0.01
Means with different superscripts in a row and column differ significantly at P≤0.01

Table 3.
Cell mediated immune response in heat stressed broilers supplemented with antioxidants.

Treatment	Increase in skin thickness (mm) against PHA-Pm—		
	4th wk	6th wk	Average+SEM
Stress free control	0.43 ± 0.009 [e]	2.06 ± 0.031 [a]	1.24 ± 0.011
Heat stressed (HS)	0.09 ± 0.009 [j]	0.26 ± 0.007 [i]	0.17 ± 0.011
HS+ Vitamin E (200 mg/kg)	0.32 ± 0.024 [h]	1.47 ± 0.052 [d]	0.86 ± 0.012
HS+ Vitamin E (200 mg/Kg)+Se (0.15mg/kg)	0.39 ± 0.011 [f]	1.82 ± 0.009 [b]	1.09 ± 0.010
HS+Tulsi (0.25%)	0.27 ± 0.006 [g]	1.65 ± 0.011c [c]	0.96 ± 0.012
HS+Tulsi (0.5%)	0.29 ±0.006 [g]	1.69 ±0.006 [c]	0.99 ± 0.012
P value	0.001	0.001	

Diet Period interaction P≤0.01
Means with different superscripts in a row and column differ significantly at P≤0.01