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## Thyroid hormones and Corticosterone investigation under heat stress in native chicken

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Chickens were highly susceptible to high environmental temperature due to lack of sweat glands and high metabolic rate which leads to increased mortality and enormous economic losses every year. Hyperthermia induces blood compositional changes, including metabolic and endocrine alterations (Habibian *et al.* 2014). Thyroid hormones were well known to be influenced by stress and act on multiple metabolic processes. Several researchers reported reduced concentrations of  $T_3$  and  $T_4$  in heat stressed chickens. The inverse relationship exists between concentration of thyroid hormones and environmental temperature, corticosterone (Iqbal *et al.* 1990).

In broilers, thermal conditioning at an early age resulted in improved thermotolerance and reduced mortality when re-exposed to heat in later life (De Basilio *et al.* 2001). Early thermal conditioning seems to be one of the most promising methods to improve the adaptability of chickens to heat stress. Hence, the objective of this study was to evaluate the effect of thermal conditioning on different native chicken strains in amelioration of heat stress and normalization of serum  $T_3$  and  $T_4$  and corticosterone levels.

The study was conducted at Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai and all experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC). Twelve weeks duration study was conducted to evaluate the effect of thermal conditioning in four different native chicken strains namely Aseel, Naked Neck (NN), Assel × Nandanam chicken-4 (ARW) and Naked Neck × Nandanam Broiler-3 (NNB3). A total of 960 chickens, i.e. 240 numbers of each variety with straight run chicks were used in the study. Chicks from four varieties (ASL, ARW, NN, NNB3) were divided into control (C) and heat exposed (H) groups. The C chicks were reared in ambient temperature  $(28\pm1^{\circ}C)$ . H group chicks were exposed to 39±1°C for 2 hrs daily during 0-2 weeks and 5-6 weeks of age, in the thermal chamber using thermostat controlled equipment. At 12<sup>th</sup> week, control group were divided into two groups, i.e. unexposed control (C) and sudden exposed control (CE).

Present address: <sup>1</sup>Madras Veterinary College, Chennai 600 007; <sup>2</sup>Poultry Research Station, TANUVAS, Chennai 600 051. ⊠Corresponding author e-mail: varunsivagangai92@gmail.com Birds from H group (HE) and CE group were thermal challenged at  $39\pm1$  °C for 4 hours daily on  $12^{th}$  week.

On  $42^{nd}$  and  $84^{th}$  day, blood was withdrawn from each group, serum separated and processed for T<sub>3</sub>, T<sub>4</sub> and CORT by commercially available Radioimmunoassay kit (Immunotech, Czech Republic) as per the manufactures recommendation. The hormone level was expressed in ng/ dl. The data collected were subjected to statistical analysis of one way ANOVA and T test as per the procedure of statistical analysis system (SPSS version 20.0 for windows).

Statistical analysis of hormone levels on thermal conditioning and thermal challenge were presented in Table 1. On 42<sup>nd</sup> day, T<sub>3</sub> and T<sub>4</sub> concentration was significantly (P<0.01) decreased in H group when compared with control (C) group in all the chicken strain. Among the strains, NN broiler had significantly low T<sub>3</sub> concentration on 42<sup>nd</sup> day. However at 84<sup>th</sup> day, sudden exposure to high temperature for longer duration (CE group), had significantly lower T<sub>3</sub> and  $T_4$  concentration, while the pre-exposed group (HE) had significantly high T<sub>3</sub> and T<sub>4</sub> levels and also comparable with C group. Metabolism in birds during growth and production was regulated by thyroid hormone; therefore it is important in adaptation of birds to heat stress. On 42<sup>nd</sup> day, the T<sub>3</sub> and T<sub>4</sub> concentration was significantly lower in heat exposed birds. Whereas, at 84th day of age the T<sub>3</sub> and T<sub>4</sub> concentration was significantly lower in CE group indicating the stress condition. However, T<sub>3</sub> and T<sub>4</sub> levels in HE group were significantly higher than CE and significantly lower than C group on 84th day, indicating that the pre-exposed birds were able to acclimatise high temperature upon re-exposure to heat stress.

Heat stress depresses the activity of the thyrotrophic axis in birds which reflected by decreased plasma  $T_3$  and  $T_4$ levels. Thermal conditioning in birds resulted in improved thermo-tolerance. Thyroxin ( $T_4$ ) is considered to be a prohormone of the more biologically active 3, 5, 32triiodothyronine ( $T_3$ ). The  $T_3$  and  $T_4$  play important roles in regulating metabolism and thermogenesis of chicken. The present findings with respect to decreased  $T_3$  levels in NN and NNB3 broiler strains were in agreement with the findings of Vinoth *et al.* (2016) reported that there was a significantly decreased  $T_3$  levels in the heat exposed Punjab

Strain	<i>Triiodothyronine</i> $(T_3)$ (ng/dl)						
	42 day			84 day			
	С	Н	t-value	С	HE	CE	F-value
Aseel	2.093 <sup>bA</sup> ±0.008	$1.963^{aB}\!\!\pm 0.009$	5.68**	$2.097^{bA} \pm 0.024$	$1.987^{aB} \pm 0.015$	1.743 <sup>aC</sup> ±0.012	105.08**
ARW	$2.117^{abA}\pm0.018$	$1.873^{bB} \pm 0.018$	9.16**	$2.127^{abA}\pm0.019$	1.963 <sup>abB</sup> ±0.009	1.693 <sup>abC</sup> ±0.009	287.36**
NN	2.163 <sup>aA</sup> ±0.009	1.793 <sup>cB</sup> ±0.022	6.41**	2.173 <sup>aA</sup> ±0.012	1.920 <sup>bcB</sup> ±0.006	1.660 <sup>bC</sup> ±0.015	480.76**
NNB3	$2.157^{aA}\pm0.024$	1.823 <sup>bcB</sup> ±0.032	9.93**	$2.180^{aA}\pm 0.015$	1.950 <sup>cB</sup> ±0.006	$1.667^{abC} \pm 0.027$	196.17**
F value	4.107*	11.581**		4.818*	8.708**	4.775*	
Thyroxine	$(T_{A})$ (ng/dl)						
Aseel	11.25 <sup>cA</sup> ±0.34	8.21 <sup>cB</sup> ±0.19	6.22**	10.75 <sup>bA</sup> ±0.29	9.23 <sup>cB</sup> ±0.32	7.81 <sup>cC</sup> ±0.10	340.4**
ARW	11.82 <sup>bcA</sup> ±0.14	$8.86^{bB} \pm 0.07$	6.34**	11.37 <sup>bA</sup> ±0.09	9.29 <sup>cB</sup> ±0.05	7.33 <sup>dC</sup> ±0.21	225.7**
NN	13.42 <sup>aA</sup> ±0.35	9.21 <sup>aB</sup> ±0.19	9.11**	12.65 <sup>aA</sup> ±0.34	11.79 <sup>aB</sup> ±0.12	9.13 <sup>bC</sup> ±0.13	395.6**
NNB3	13.17 <sup>abA</sup> ±0.32	9.51 <sup>aB</sup> ±0.04	9.64**	12.52 <sup>aA</sup> ±0.14	$10.27^{bB}\pm0.08$	$9.60^{aC} \pm 0.26$	615.9**
F value	11.81**	25.11**		14.6**	148.72**	376.17**	
Corticoste	erone (ng/dl)						
Aseel	8.13 <sup>cB</sup> ±0.05	22.34 <sup>cA</sup> ±0.41	40.24**	8.56 <sup>bC</sup> ±0.16	17.65 <sup>bB</sup> ±0.19	23.86 <sup>cA</sup> ±0.26	535.59**
ARW	8.92 <sup>bB</sup> ±0.07	23.41 <sup>bcA</sup> ±0.43	42.94**	$8.91^{abC} \pm 0.08$	$18.14^{bB}\pm0.16$	24.53 <sup>bA</sup> ±0.29	710.55**
NN	$9.27^{aB}{\pm}0.08$	25.13 <sup>aA</sup> ±0.26	45.06**	9.21 <sup>aC</sup> ±0.13	$19.74^{aB}\pm0.18$	$25.79^{aA}\pm 0.07$	281.63**
NNB3	9.31 <sup>aB</sup> ±0.04	24.02 <sup>abA</sup> ±0.21	47.71**	9.12 <sup>aC</sup> ±0.02	19.55 <sup>aB</sup> ±0.41	24.63 <sup>bA</sup> ±0.29	352.16**
F value	75.28**	11.76**		6.83*	16.51**	10.76**	

 Table 1. Effect of thermal conditioning and thermal challenge on Triiodothyronine (T3), Thyroxine (T4) and Corticosterone (ng/dl) in different chicken strains (Mean ± SE)

C, Control; H, Thermal Conditioned ( $39\pm1^{\circ}C-2h$ ); CE, Control exposed (84 d;  $39\pm1^{\circ}C-4 h$ ); HE, Heat Exposed (84 d;  $39\pm1^{\circ}C-4 h$ ). ARW, Aseel × Nandanam Chicken-4; NNNB-3, Naked Neck × Nandanam Broiler-3; NN, Naked Neck; AT, Ambient temperature. <sup>a,b,c</sup>Means with different superscript within same columns have significant differences. <sup>A,B,C</sup>Means with different superscript within same rows have significant differences. NS, Non-significant; \*, Significant (P<0.05); \*\*, Highly significant (P<0.01).

broiler-2 birds. Liang *et al.* (2016) observed the plasma  $T_3$  levels was significantly decreased in heat exposed birds. The present findings were in disagreement with Rajkumar *et al.* (2015) who reported that a non-significant difference exists between the heat exposed and unexposed group with respect to  $T_3$  levels. The importance of thyroid hormones in adaptation to heat stress was related to its role in regulation of the metabolic rate in birds during growth and production.

On 42<sup>nd</sup> day, CORT concentration was significantly (P<0.01) elevated in H group when compared with control (C) group. Among the strains, NNB3 and NN had significantly (P<0.05) elevated CORT levels. However at 84<sup>th</sup> day CE group, sudden exposed to high temperature for longer duration had significantly (P<0.01) elevated CORT level, while the pre-exposed group (HE) had significantly low CORT level when compared to CE group but significantly high when compared to control (C) group. Among the varieties, NNB3 and NN had elevated CORT level.

Corticosterone level was used as an indicator to access the quantum of stress. The present findings with respect to increased CORT in NN and NNB3 broiler strain were in agreement with the findings of Rimoldi *et al.* (2015) who recorded increased CORT level in the heat exposed birds when compared with the control birds. However, the present findings were in disagreement with the findings of El-Azim (2012) who showed that a non-significant difference exists between the heat exposed and unexposed group with respect to CORT level. This difference in corticosterone value among different strains might be due to differences in the heat exposed period and genetic makeup of the groups used in the study.

Heat stress is one of the factors that activate the hypothalamic-pituitary-adrenal (HPA) axis. The stress activated HPA axis, leads to rapid release of corticotropinreleasing hormone (CRH) and adreno-corticotropic hormone (ACTH) from the cells located in the hypothalamus and pituitary, respectively. ACTH stimulates the synthesis and release of steroids from the adrenal cortex, namely corticosterone (Jahejo et al. 2016). The birds exposed to high temperature, which causes the release of corticosterone into the blood circulation, which will aid in the metabolism of the birds. The lower corticosterone level in the thermal conditioned groups suggests that the birds were successfully habituated to the heat stress. It was also found that the blood CORT concentration is negatively correlated with feed intake, which will help to reduce further heat increment by digestion and metabolism.

From the present study it was noticed that that thyroid and corticosterone hormones were influenced by thermal conditioning, which can be used as an indicator to assess the quantum of heat stress. It was also noted that the thermal conditioning leads to gradual acclimation to long term heat exposure which was indicated by drop in corticosterone level.

## SUMMARY

The study was conducted to evaluate the effect of thermal conditioning on serum levels of Triiodothyronine  $(T_3)$ ,

thyroxine  $(T_4)$  and corticosterone (CORT) in four different chicken strains namely Aseel, Naked Neck, Assel × Nandanam chicken-4 and Naked Neck × Nandanam Broiler-3. Chicks were divided into control (C; ambient temperature 28±1°C) and heat exposed groups (H; 39±1°C for 2 hours; 0-2 and 5-6 weeks of age). At 12<sup>th</sup> week, control group were divided into two groups, i.e. unexposed control (C) and exposed control (CE). Birds from H group (HE) and CE group were thermally challenged at 39±1°C for 4 hours daily on 12th week. On 42nd and 84th day, blood was withdrawn from each group, serum separated and processed for T<sub>3</sub>, T<sub>4</sub> and CORT. The results indicated that the thermal conditioning had significantly decreased  $T_3$ , T<sub>4</sub> and increased CORT levels, irrespective of strains. At thermal challenge, a drastic drop in CORT level and improvement in thyroid hormone levels were noticed in the preconditioned birds. From present study, it was concluded that, the birds are able to withstand the heat stress effect which was indicated by drop in CORT level.

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