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Effect of heat stress on haemato-biochemical and corticosterone level of coloured synthetic broiler male line

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Abstract
The present study aimed to determine haemato-biochemical constituents and corticosterone level of synthetic coloured broiler male line affected with heat stress. We hypothesized that haemoglobin (Hb), Packed cell volume (PCV), total erythrocyte count (TEC), lymphocyte and eosinophil values were significantly higher in control whereas total leucocyte count (TLC), heterophil and basophil were significantly higher in heat stress group. Additionally, Heterophil/Lymphocyte (H/L) ratio in heat stress group was observed to be highly significant (p<0.01). Biochemical pictures depicted significantly higher (p<0.01) levels of glucose and aspartate aminotransferase (AST) in heat stress group as compared to control whereas values of total protein, albumin, globulin and alanine transaminase (ALT) were showing no significant difference between these two groups. When compared to the control group, the corticosterone level in the heat stress group was found to be greater (p<0.01).

Keywords: Broiler, corticosterone, glucose, heat stress, heterophil

Introduction
Coloured broiler breeders are becoming popular in recent years because of the preference for a range of poultry products as well as the welfare issues in the poultry industry. Global warming is one of the major challenges faced by the poultry industry affecting the performance of the birds (Gregory 2010) [12]. Stress evokes harmful responses that interferes with the general health, productivity and result in immunosuppression (Saxsena and Madan 1997) [31]. High temperature imposes severe stress on birds and leads to important economic losses in the poultry. Although birds perform well within a relatively wide range of temperatures, between 10 and 27 °C, temperatures above 30 °C may cause stress in adult hens (Daghir 2009) [9] and broiler chickens (Geraert *et al.*, 1996) [10]. Broiler needs the ambient temperature in a range that is appropriate to live in comfortable conditions, which is known as thermo neutral zone. If broiler is reared above the condition of thermo neutral zone, it will be vulnerable to environment stress (Ahmed *et al.*, 2008) [2] and exhibit behavioral and physiological changes (Attia *et al.*, 2017) [6]. Lin *et al.*, (2006) [20] and Shekhar *et al.*, (2018) [32] mentioned that if the thermoregulation mechanism is insufficient to maintain homeothermy, the body temperature begins to rise and eventually cause death from heat stress. Heat loss by evaporative heat dissipation is linked to relative humidity of the surrounding environment (Lara and Rostagno 2013) [19]. Therefore, high temperature accompanied by high humidity is more detrimental to broiler performance than high temperature with low humidity. The evaporative heat loss increases along with temperature and decreases with increasing humidity (Lin *et al.*, 2006) [20].
Determination of haematological parameters or indices has been a reliable tool as blood often gives specific indication of the ongoing events in the body, serving as an aid to diagnosis and health status assessment (Tibbo *et al.*, 2004) [35]. A number of hematological indices such as haematocrit value, hemoglobin concentration, red blood cells count and so on, are used to assess the functional status of the oxygen carrying capacity of the blood stream (Maheswaran 2008) [21]. Factors such as age, gender, environmental factors including season and stress to which the animal has been exposed have been shown to influence haematological parameters (Olayemi and Arowolo 2009) [25]. By evaluating the haematological profile during the seasonal variations, the confusion with disease associated changes can be avoided. Blood biochemical profile such as glucose, total protein, aspartate aminotransferase (AST), alanine

aminotransferase (ALT) are of diagnostic values for various disease conditions and having particular reference to liver disorders, kidney diseases, diarrhea, dehydration, etc. (Akporhuarho 2011) [3].

As the information on haemato-biochemical profile of domesticated avian species in summer seasons are scanty; the purpose of the present study was to determine the effects of summer stress on the haemato-biochemical parameters and on corticosterone level in serum of coloured synthetic broilers male line.

Materials and Methods

The research work was conducted in the Poultry science department, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha. The prior ethical approval from the Institutional Animal Ethics Committee (IAEC), C.V.Sc & A.H., OUAT, Bhubaneswar, Odisha- 751003 was obtained for the use of birds in this study, with humane care.

Calculation of Temperature humidity Index

Due to its relative simplicity and the accessibility of the meteorological data utilised in its formulation, THI was employed in this study to assess heat stress conditions for broilers. The THI formulas were taken from Cicero *et al.* (2018) [8], who claimed that for the poultry indices, maximum daily temperature (Tmax) and minimum daily temperature (Tmin) might be used in place of Tdb and Twb, respectively.

THI (broiler) = 0.85 Tmax + 0.15 Tmin

Experimental design

The trial was conducted from March to June, 2019. A total of forty coloured synthetic broiler male line were reared under uniform husbandry conditions. Daily temperature and humidity were recorded and based on THI value, twenty birds at the age of 19-22 weeks with average body of 2.2 kg were kept in control group from March-April (2019) whereas twenty birds at the age of 28-32 weeks with average body of 2.75 kg were kept in heat stress group from May-June (2019).

Management of birds

Identical care and management were provided to birds of both groups throughout the experimental period. Clean, fresh water and commercial broiler feed was provided containing 18% CP and 2600 ME throughout the experimental period. The experimental diets were analyzed for proximate composition as per AOAC [5].

Sample collection

For haematological studies 5ml blood were taken from the brachial vein in EDTA coated vials whereas for biochemical and corticosterone studies, blood samples were collected without anticoagulant to prepare serum. Samples for control group were collected from each bird between 09:00 AM. to 10:00 AM. in month of March-April whereas 03:00 to 04.00 P.M. was selected in month of May-June for heat stress group.

Haematological studies

Haemoglobin amounts were estimated by modified acid haematin method by using Sahli’s haemoglobinometer whereas PCV was measured by hematocrit tube centrifuged at 2000 rpm for 20 min. For differential leukocyte counts Wright-Giemsa staining procedure was followed. TLC and TEC estimation has been done by using Natt Herrick solution. Erythrocyte indices viz, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were analyzed by using standard formulae.

Biochemical estimation

Glucose, total protein, albumin, globulin, ALT and AST in serum has been estimated through commercial diagnostic kits (Coral Clinical System) by procedure mentioned in it.

Corticosterone estimation

Serum corticosterone level was assayed by chicken corticosterone competitive ELISA kit (Cat No. #*E0098ch*, *Bioassay Technology* Laboratory, China) as described by manufacturer.

Statistical analysis

The different haemato-biochemical parameters and serum corticosterone level in control and heat stress group were statistically analyzed using Statistical Package for Social Science (SPSS) version 25, Windows 10.0. Descriptive statistics in the form of mean ± standard error was used to measure all the parameters. The significance (P-value) was recorded at 5% ($p<0.05$) and 1% ($p<0.01$) level.

Results

Temperature, Relative Humidity and THI

As indicated in Table-1, the mean Tmax and Tmin values for the control group were 29.55±0.27 and 19.15±0.24 respectively, but for the heat stress group, these values were 34.55±0.21 and 22.6±0.21 respectively. However, the average THI in the control and heat stress group was 27.99±0.22 and 32.75±0.18 respectively.

Haematological Parameters

As shown in Table- 2, the haematological findings revealed that the value of Hb, PCV and TEC in control group were significantly ($p<0.05$) higher than that of heat stress group. Simultaneously TLC value was significantly ($p<0.05$) lower in control than that of affected ones.

Erythrocyte indices viz, MCV (fL), MCH (pg), MCHC (g/ dl) obtained in this study were statistically non-significant between control and heat stress group. The mean value of heterophil, basophil percentage and H/L ratio were significantly ($p<0.01$) higher in heat stress compared to control whereas lymphocyte and eosinophil counts are significantly lower in heat stress group. On the other hand, monocyte counts did not differ statistically.

Biochemical Parameters

The results of study on biochemical parameters between control and heat stress group had been presented in Fig. 1 and Fig. 2. The mean serum total glucose level in heat stress group were found to be significantly higher ($p<0.01$) than control. Value of total protein, albumin, globulin and ALT were found to be statistically non-significant between control and heat stress group. In addition, the AST activity was significantly ($p<0.01$) higher in heat stress group.

Serum Corticosterone Level: As in Fig. 3, corticosterone was significantly higher ($p<0.01$) in heat stress group as compared to control.

Discussion

THI

The value of THI in both control group and heat stress group was in agreement with the findings of (Marai *et al*., 2001) [22] who demonstrated the corresponding values for non-sweating animals such as poultry and pigs are as follows: normal < 27.8, moderate 27.8–28.8, severe 28.9–29.9 and very severe (emergency) ≥ 30.0.

Haematological Parameters

Hb, PCV and TEC values in the control group were significantly higher than those in the heat stress group as indicated in Table-2. These findings were consistent with (Nadia 2003) [23] and (Gharib *et al*., 2005) [11]. Hemodilution factor, an adaptive response that permits water loss by evaporation without affecting plasma volume, may be the cause of a large reduction in Hb and PCV. While a rise in ambient temperature during the summer could be the cause of a fall in the TEC value, which eventually causes an increase in avian body temperature, respiration, and oxygen consumption as per Brackenbury *et al*., (1981) [7]. Because of the increased oxygen intake, the partial pressure of oxygen in the blood decreases erythropoiesis and as a result, the number of circulating erythrocytes decreases. Altan *et al*., (2000) [4] hypothesized that a temperature high enough to affect alterations in circulating leucocyte components in chicken. The current findings of higher TLC in the heat stress group support their hypothesis.

The current study's low value of MCH and MCHC during summer agreed with (Olayemi and Arowolo, 2009) [25] who hypothesized that these results could be due to changes in blood volume and viscocity. Increased heterophil, decreased lymphocyte and altered H/L ratio have been proposed as sensitive and reliable measures of stress in broiler (Gross and Sigel, 1983) [13]. According to (Khan *et al*., 2002) [17] the decrease in lymphocyte number during thermal stress could be due to a reduction in the size of lymphatic organs. The increased H/L value agreed with (Altan *et al*., 2000) [4] and (Huff *et al*., 2005) [15] who stated that changes in haematological values of the H/L ratio are a good indicator of stress. Aengwanich and Chinrasri (2003) [1] claimed that the H/L ratio assesses the physiological change in organs like the thymus and bursa of fabricious atrophy that is altered by the action of corticosteroids as corticosteroids trigger the release of heterophils.

Biochemical Parameters

The mean serum total glucose level in the heat stress group was significantly higher which was consistent with the findings of (Shim *et al*., 2006) [33], (Olanrewaju *et al*., 2010) [26] and (Rashidi *et al*., 2010) [29]. Kolb (1984) [18] discovered that an increase in glucocorticoid concentration responds predominantly to an increase in glucose because glucocorticoids stimulate gluconeogenesis from muscle tissue proteins, lymphoid and connective tissue. The amount of total protein in the heat stress group was significantly lower than control which was consistent with the findings of (Sahin 2002) [30]. The heat stress group had significantly higher AST activity which was supported by (Khan *et al*., 2002) [17] and (Ramnath *et al*., 2007) [28]. Hsueh *et al*., (2011) [14] and (Oche *et al*., 2014) [24] proposed that when the integrity of the hepatocellular membrane, muscles and cardiac muscle fibres is compromised, markers enzyme is extruded into the blood stream.

Serum Corticosterone Level

Blood corticosterone concentration is the most sensitive indicator of stress in broilers as per (Puvadolpirod *et al*., 2000) [27]. Thaxton *et al*., (2004) [34] discovered that when chicken's wellbeing is jeopardized by stressful conditions, corticosterone levels in their blood rise. A significant increase in serum corticosterone in heat stress group indicated that birds were stressed as was observed in mammals for cortisol (Kataria *et al*., 2008) [16]. It was accompanied by increased concentrations of energy nutrients in sera like glucose which substantiated the significance of corticosterone in meeting the energy crisis during stress.

Table 1: Mean (±SE) values of ambient temperature, relative humidity and THI during experimental period.

Month	Ambient temperature (°C)		Relative humidity (%)	THI
	Maximum(Tmax)	Minimum(Tmin)		
Control Group (March-April)	29.55±0.27	19.15±0.24	62.7±0.47	27.99±0.22
Heat stress Group (May-June)	34.55±0.21	22.6±0.21	77.25±0.57	32.75±0.18

Table 2: Haematological profile (Mean±S.E) in coloured synthetic broiler

Sl. No.	Parameters	Control group (March-April)	Heat stress Group (May-June)	P value	t- value
1	Hb(g/dl)	9.49^a±0.16	8.09^b±0.13	0.000	5.844**
2	PCV (%)	31.45^a±1.09	28.20^b±0.43	0.014	2.702*
3	TEC($x10^6$/µl)	2.78^a±0.08	2.44^b±0.08	0.003	3.445**
4	TLC($x10^3$/µl)	26.90^b ±0.72	29.90^a ±0.86	0.012	2.775*
5	H: L Ratio	0.34^b±0.03	0.46^a±0.01	0.001	3.936**
6	MCV (fl)	114.16±3.65	117.92±4.10	0.437	0.794
7	MCH (pg)	34.80±1.22	33.91±1.38	0.582	0.560
8	MCHC (g/dl)	30.86±1.16	28.79±0.6	0.163	1.453
9	Heterophil	23.35^b±1.40	28.9^a±0.47	0.004	3.312**
10	Lymphocyte	70.45^a±1.18	62.75^b±0.57	0.000	5.833**
11	Eosinophil	3.25^a±0.28	2.40^b±0.2	0.037	2.243*
12	Basophil	1.55^b±0.21	4.75^a±0.23	0.000	10.514**
13	Monocyte	1.85±0.22	1.2±0.24	0.055	2.041

* Means with different superscripts (a, b) differ significantly between groups ($p<0.05$)
**Means with superscripts (a, b) differ significantly between groups ($p<0.01$)

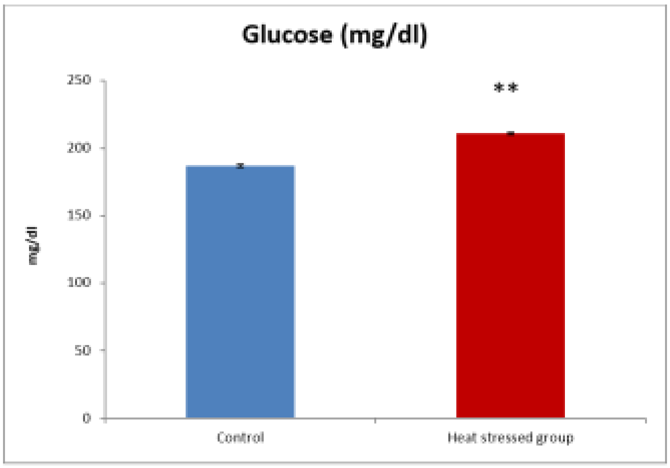

Fig 1: Concentration of Glucose (mg/dl) in control and heat stressed group. Values are shown as mean±SE. ** denotes statistically significant between groups ($p<0.01$)

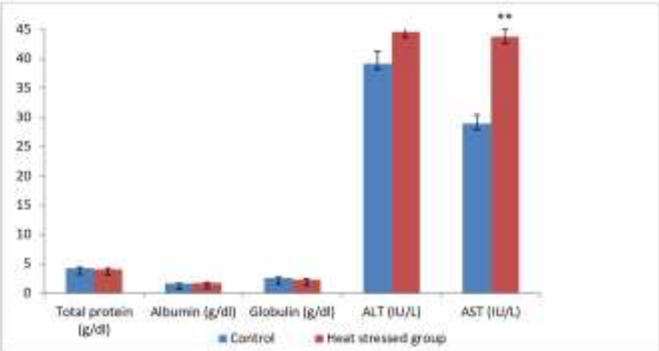

Fig 2: Concentration of total protein, albumin, globulin, ALT and AST in control and Heat stressed group. Values are shown as mean±SE. ** denotes statistically significant between groups ($p<0.01$)

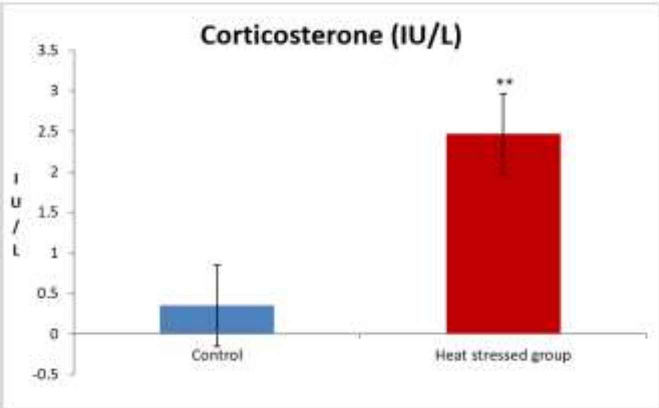

Fig 3: Concentration of corticosterone (IU/L) in control and heat stressed group. Values are shown as mean±SE. ** denotes statistically significant between groups ($p<0.01$)

Conclusion

The results of this study and the data presented here may significantly improve researchers' comprehension of the haemato-biochemical and corticosterone levels of coloured synthetic broilers under heat stress circumstances.

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