



Sexual difference in chicken blood parameters during high ambient temperature

M. SHANMUGAM*, T.K. BHATTACHARYA, M.R. REDDY, S.V. RAMA RAO AND U. RAJKUMAR

Project Directorate on Poultry, Rajendranagar, Hyderabad-500 030, India

(Received on December 05, 2016; accepted for publication on August 31, 2017)

ABSTRACT

Shanmugam, M., Bhattacharya, T.K., Reddy, M.R., Rao, S.V. Rama and Rajkumar, U. 2017. Sexual difference in chicken blood parameters during high ambient temperature Indian Journal of Poultry Science, 52(2): 225-227.

The present study was carried out to record the status of different blood and serum biochemical parameters of White Leghorn chicken at high ambient temperature. A total of 18 birds (9 birds of each sex) of 20 weeks age were used in the study. The heterophil:lymphocyte (H:L) ratio indicated that the birds were experiencing heat stress due to high ambient temperature. Significant difference was observed between the sexes in haemoglobin, total erythrocyte count, heterophils, H:L ratio, calcium and lipid peroxidation levels. Results of this study revealed that high ambient temperature induces stress that affects the sexes differently in chicken as evident from their blood profile.

Key words: Heat stress, Blood, Serum, Biochemical, WLH chicken

The extreme environmental conditions impact stress that stimulate body responses to maintain homeostasis in the birds. Abiotic stress factors like high temperature and humidity are major stressors in poultry production under tropical environment. A temperature range of 5-24°C is adaptable and tolerable by hen (Sanisbury, 2000) and the bird's temperature regulating mechanism evinces difficulty when environmental temperature is higher than 32.2°C (Oguntunji and Alabi, 2010). The relative humidity becomes important and affects production when the ambient temperature is more than 28°C and the preferred relative humidity range is 60-65% (Yahav, 2000). The reproductive efficiency of heat stressed White Leghorn chicken is affected in comparison to those hens maintained at thermo neutral environment of 24 to 26°C (Rozenboim *et al.*, 2007). The reproductive efficiency along with other performances in the chicken is affected due to various physiological changes like change in blood composition and flow dynamics (Wolfenson, 1986). Plasma cholesterol concentration was found to be increased in heat stressed broiler chicks (Kutlu and Forbes, 1993). High ambient temperature causes an increased production of malondialdehyde (MDA) a product of lipid peroxidation in serum of laying hens (Naziroglu *et al.*, 2000). Heat stress causes a disturbance of the equilibrium between the generation and reduction of oxidants and hence increases oxidative stress in broiler chickens (Lin *et al.*, 2006). It has been suggested that acclimatization occurs during slow seasonal changes and constant exposure to cyclic temperatures (Yahav, 2009). There is lack of information on different blood parameters of layer chicken at high ambient temperature under tropical environmental conditions and the response of sexes during these conditions. Therefore the present study was undertaken to know the sexual differences in

blood physiological status of White Leghorn chicken, under high ambient temperature.

The present study was conducted at the experimental farm of Project Directorate on Poultry, Rajendranagar, Hyderabad, India. The experiment was carried out during the month of April and the average high and low shed temperature recorded during the week of blood collection was 37.2°C and 27.5°C respectively and relative humidity 56.3% and 30.7% respectively. The THI during the week of the study was 80.11 and was calculated according to the formula: $THI = (0.8 \times Ta) + [(RH / 100) \times Ta - 14.3] + 46.4$ (Mader *et al.*, 2010). A total of 18 White Leghorn chicken (9 birds of each sex) of 20 weeks age were selected randomly for the experiment and housed in individual cages in an open sided house under standard management procedures. The female birds had started egg production at the time of the experiment. Birds were provided with *ad libitum* feed and water. The birds received a layer diet containing 2650 metabolizable energy (ME) kcal/kg and 17.0% crude protein. The calcium level in the diet was 3.7% for females and 1.0% for males.

Blood was collected from each bird from the wing vein into two vials, one with anticoagulant (EDTA) and the other without anticoagulant for serum separation. Serum was separated by centrifuging the vials at 2500 x g for 10 min within 2 hours of blood collection and samples were stored at -20°C till further analysis. Whole blood was used for the estimation of complete blood picture and heterophil:lymphocyte (H:L) ratio.

Serum samples were analyzed for protein by Lowry *et al.* (1951) method, cholesterol by the method described by Zak *et al.* (1954) and calcium was estimated using a commercial kit (o-cresophenolphthalein complexone method) (Nice Chemicals, Cochin). Nitric oxide (NO) in serum was determined by the method described by

*Corresponding Author Email: dr_shan@rediffmail.com

Miranda et al. (2001). Briefly, the samples were deproteinised by treating with equal volume of ethanol and centrifugation at 3000 rpm for 10 minutes. The supernatant was used for total nitrite determination by Griess assay after conversion of nitrate to nitrite by Vanadium (III) chloride reduction and the colour intensity was measured at 540 nm in an ELISA reader. Lipid peroxidation (LP) in the serum was assessed by the protocol described by Placer et al. (1966). Briefly, 200 µl serum was mixed with 1.3 ml of Tris KCl buffer and 1.5 ml of TBA reagent and boiled for 10 minutes in a water bath. The reagents were cooled and then added 3 ml of pyridine-butanol (3:1) mixture and 1 ml of 1N NaOH. Absorbance of test samples was measured at 548 nm against blank and the total amount of lipid peroxidation was calculated in terms of nmol malondialdehyde (MDA)/mg protein.

Blood and serum biochemical parameters were analysed following Student's 't' test to explore the effect of sex using SPSS 10.0 software.

The overall values of blood parameters obtained during the experiment were presented in Table 1. Analysis of data grouped on the basis of sex indicated that there is significant difference between the sexes in haemoglobin and total erythrocyte count. The heterophils percentage as well as H:L ratio was found to be statistically significant and higher in female birds than males. The serum biochemical parameters were not significantly different between the sexes except in case of calcium and LP levels (Table 2).

The haemoglobin (Hb) and total erythrocyte count

(TEC) values are within the normal reported range. The H:L is a sensitive indicator of stress, and respectively 0.2, 0.5, and 0.8 characterize low, optimum, and high levels of stress (Gross and Seigel, 1983). The combined H:L ratio obtained in the present study indicates that the birds are experiencing stress due to high environmental temperature which is in agreement with other earlier reports. Laying hens were found to be heat stressed under cyclic daily temperatures and humidity ranging from 23.9 to 35°C and from 50 to 15% representing natural cyclic temperatures during hot summer months (Mashaly et al., 2004). A threshold THI of 70 was reported for chicken (Karaman 2007). In the present study the calculated THI was higher compared to that of the threshold value for chicken. Therefore the period of study should have been stressful to the experimental birds. The difference in the Hb and TEC between the sexes might be due to the physiological difference. The higher H:L ratio in females clearly indicates that they are experiencing more stress than males during high ambient temperature.

Higher serum calcium level in females is due to the high calcium in the diet. Diurnal cyclic temperature (15:35°C) did not influence the haemoglobin level in male broiler chickens. However the plasma total protein was significantly elevated during the 35°C phase of the experiment (Yahav et al., 1997). In our study there was no significant difference in cholesterol level between the sexes indicating that there was no effect of heat stress or sex on this parameter. In contrast Saeki (1971) observed in four breeds of chicken studied males had significantly lower plasma cholesterol level than that of

Table 1: Mean±SE values of haematological parameters in the IWI strain of White Leghorn chicken at high ambient temperature

Parameter	Male	Female	Overall	P Value
Hb (%)	9.57±0.24 ^a	8.04±0.40 ^b	8.81±0.29	0.006
TEC (X10 ⁶)	3.02±0.09 ^a	2.70±0.11 ^b	2.9±0.07	0.037
Basophills (%)	1±0	1.25±0.25	1.08±0.08	0.391
Eosinophills (%)	1.44±0.29	2.11±0.65	1.77±0.36	0.373
Monocytes (%)	1.89±0.35	1.78±0.28	1.83±0.22	0.807
Lymphocytes (%)	62.33±2.14	58.0±2.10	60.20±1.54	0.165
Heterophills (%)	32.55±1.22 ^b	38.0±1.62 ^a	35.28±1.19	0.017
H:L Ratio	0.51±0.03 ^b	0.66±0.06 ^a	0.59±0.04	0.032

Figures bearing different superscripts in a row differ significantly

Table 2: Mean±SE values of serum biochemical parameters in the IWI strain of White Leghorn chicken at high ambient temperature

Parameter	Male	Female	Overall	P Value
Protein (g/dL)	5.55±0.18	6.46±0.45	6.01±0.26	0.088
Cholesterol (mg/dL)	137.66±7.70	161.16±14.68	150.10±8.82	0.182
Calcium (mM/L)	1.24±0.16 ^b	3.51±0.32 ^a	2.37±0.33	0.000
Nitric Oxide (mM/L)	133.16±10.12	172.23±15.81	152.70±10.27	0.057
Lipid Peroxidation (moles of MDA/g Protein)	1.22±0.13 ^b	5.63±0.63 ^a	3.55±0.64	0.000

Figures bearing different superscripts in a row differ significantly

laying hens. The egg cholesterol is synthesized in liver, transported in blood and is then incorporated in the egg yolk. The non significant finding in serum cholesterol between the sexes may be because not all the females have started egg production and only a numerically higher cholesterol value is observed in the females. Incidentally, LP level an indicator of oxidative stress was observed to be significantly different between the sexes suggesting that high ambient temperature has differing effect on the sexes. Heat stress causes disruption of balance between free radical production and the antioxidant system in laying hens (Wang *et al.*, 2002). This might be due to the production performance of hens where high level of metabolism and oxygen utilisation takes place. The NO level was not significantly different between the sexes but numerically female birds had higher level. There was no change in plasma NO level when five-day-old chicks were exposed to heat stress and there was no correlation between body temperature and plasma/diencephalon NO level (Coleone *et al.*, 2009). However, the higher level of NO in this study when observed with the high significant level of LP implies that it might have contributed to the oxidative stress and resultant lipid peroxidation.

In conclusion the present study reported the haematological and serum biochemical status of White Leghorn chicken under high ambient temperature. The birds were experiencing heat stress under the study conditions. The susceptibility to heat stress is different between the sexes and therefore amelioration strategies have to be different for the sexes considering the higher production performance of female chicken.

ACKNOWLEDGMENTS

The experiment was carried out under the network project on climate change (NPCC) funded by Indian Council of Agricultural Research. Assistance in lab analysis by Mr. K. Rakesh is acknowledged.

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