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Evaluating the Immune Response and Antioxidant Potential in Four Broiler Strains under Chronic High Ambient Temperature

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ABSTRACT

The present study was carried out to evaluate the effects of thermoneutral (TN) and high ambient temperature (HAT) on immune response and oxidative status of four broiler strains. Day old broiler chicks (n=242) of four different commercial strains (Ross, Hubbard, Cobb and Arber Acer) were placed in brooding room in first two weeks and then chicks were divided into two groups: TN and HAT zones. Chicks in TN group were housed at constant room temperature, while chicks in HAT zone were kept at high ambient temperature. Chicks in each group were further divided into four sub groups. Each sub group was further subdivided into four replicates having ten chicks per replicate. Blood was collected on weekly basis from day 21 to 42. Mean serum antibody titer against Newcastle disease (ND) and serum paraoxonase (PON1) were significantly higher ($P< 0.05$) in TN zone, while serum malondialdehyde (MDA) was significantly ($P< 0.05$) higher in HAT zone. No significant differences were recorded in serum antibody titer against ND, PON1 and MDA among different broiler strains in TN zone, while in HAT zone, Ross and Arber Acer showed significantly ($P<0.05$) higher serum antibody titer against ND. In HAT zone, significantly ($P< 0.05$) lower serum MDA was found in Ross and Arber Acer. Based on these results, it was concluded that chronic high ambient temperature has negative effect on broiler immune and oxidative status. Ross and Arber Acer strains were more tolerant to summer high ambient temperature of tropical areas than Cobb and Hubbard.

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ZR conducted the study. NC and SK designed the study. RUK analysed sample and edited the paper.

Key words
Broiler strains, Antibody titer, Paraoxonase, Malondialdehyde.

INTRODUCTION

In tropical and subtropical countries, heat stress of summer seasons is a major stressor in poultry industry that produces a wide range of physiological and behavioral responses (Khan *et al*., 2011). Heat stress induced losses are the major concerns of the hot regions of the world. Heat stress reduces feed intake, weight gains and increases mortality in poultry industry (Chand *et al*., 2016). Stressful condition stimulates the production of reactive oxygen species (ROS) excessively, causing oxidative damage to protein, lipid and nucleic acid (Khan *et al*., 2011). Physiological and biochemical changes associated with high temperature accelerate ROS formation (Chand *et al*., 2016; Naz *et al*., 2016). High concentration of ROS causes oxidative injury and severe cellular damage (Khan *et al*., 2011; Alzawqari *et al*., 2016; Alhidary *et al*., 2016). Different antioxidant enzymes such superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and paraoxonase (PON1) are very important in scavenging free radicals and their metabolites to maintain normal cellular physiology (Khan *et al*., 2014a; Shah *et al*., 2016). Heat stress causes oxidative stress and increases malondialdehyde (MDA) and decreases PON1 activities (Chand *et al*., 2016).

Over the last several decades, genetic selection for optimum performance in term of faster growth, better feed efficiency and higher disease resistance are considered very important for commercial poultry production (Khan, 2011; Khan *et al*., 2016; Abudabos *et al*., 2017). Birds usually adopt themselves to the changing environmental conditions by redistributing the body reservoirs of protein and energy which may negatively affect the growth and reproduction. Heat stress stimulates the catecholamine to prepare birds for fight and flight response during heat stress, which releases the glucose. During the fight against the heat stress, if the bird is not able to cope with the heat, the exhaustive phase leads to disturbance in the homeostatic mechanisms resulting in the death of the birds (Khan *et al*., 2012a). Determination of immune status is a common indicator in birds to find the health status (Khan

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et al., 2014b) especially during the stressful condition. The interaction between genotype and environment exerts a major effect on the suitability of a strain of broiler in a particular area of the world (Khan *et al*., 2012a). High environmental temperature affects the immune system of broiler causing a reduction in antibody production (Chand *et al*. 2014).

The objective of the present study was to investigate the immune response and antioxidant status of four commercial broiler strains (Ross, Hubbard, Cobb and Arber Acres) reared in thermo neutral (TN) and high ambient temperature (HAT).

Table I.- Ingredient composition of starter and finisher diets.

Ingredient name (%)	Starter	Finisher
Corn	58	60
Poultry byproduct meal	4	4
Rice broken	0	4
Canola meal	8	10
Sunflower meal	5	3
Corn gluten	2	2
Rapeseed meal	2	0
Soybean meal	15	13
Soybean oil	0	1
Guar meal	3	0
Bone meal	0.5	0.5
Dicalcium phosphate	0.5	0.8
Sodium chloride	0.2	0.2
Lysine HCl	0.2	0.2
Lysine Sulphate	0.42	0.3
Vitamin mineral pre mix	0.3	.5
L-Threonine	0.05	0.05
DL Methonoine	0.12	0.1
Lime stone	0.8	0.8

MATERIALS AND METHODS

Experimental design and birds' husbandry

The experiment was conducted in a completely randomized design with two factors (temperature zones) and four different broiler strains (Ross, Hubbard, Cobb and Arber Acres). A total of two hundred and forty day old broiler chicks of four commercial broiler strains of the same age and size were used in the study. In the first two weeks, chicks were placed in a brooding room and then divided into two groups: TN and HAT zones. Chicks in TN zone were housed at constant room temperature (25ºC ± 2Cº and RH 65 ±5%), while chicks in HAT zone were kept at high ambient temperature. Both the groups were kept in two different rooms which were identical in term of size, construction materials and equipments. The temperature and humidity were recorded after every four hours in the centre of the house and minimum and maximum values of each day was averaged per week. Chicks in each group were further divided into four sub groups. Each sub group was further subdivided into four replicates having ten chicks per replicate. Feed and water were provided *ad libitum*. During the first 21 days, chicks were provided with starter ration containing 22% crude protein (CP) and 3000 kcal/kg metabolizable energy (ME). During the last three weeks, chicks were provided with finisher ration containing 20% CP and 3200 Kcal/kg ME. The feed composition and proximate analysis are given in Tables I and II, respectively.

All birds of four strains were vaccinated with Newcastle vaccine specified schedule.

Table II.- Chemical composition (nutritive values) of starter and finisher ration.

Ingredient name	Starter	Finisher
Crude protein (%)	22.00	20.00
Crude fiber (%)	5.27	4.58
Ether extract (%)	4.28	4.17
Calcium (%)	0.88	0.84
Phosphorus (%)	0.44	0.42
Lysine (%)	1.35	1.21
Methonine and Cysteine (%)	0.94	0.84
Threonine (%)	0.87	0.80
ME/Kcal/kg	3000	3200

Temperature and humidity data

House temperature and humidity were recorded after every four hours in the centre of the house at an interval of four hours (08:00 am, 12:00 pm, 4:00 pm, 8:00 pm, 12:00 am, and 4:00 am) (Table III).

Collection of serum and analysis

Blood samples were collected on day 21, 28, 35 and 42 from randomly selected birds (two birds per replicate) for biochemical analysis. Blood was collected from the wing vein with the help of a disposable syringe. Blood samples were centrifuged at 2,000 rpm for 10 min for serum separation. Antibody titer of ND was determined through ELISA test. The PON1 activity was determined with 2 mmol/L of paraoxon (Sigma Chemical Co., London, UK) and measured spectrophotometrically. The MDA level was determined by the method described by Chand *et al*. (2016).

Table III.- Mean temperature and relative humidity during the experimental period.

Hour	Ambient temp. (C)	Relative humidity (%)
08:00 am	31.66 ±0.94	51.65 ± 0.32
12:00 pm	36.43±0.12	52.29 ±0.91
04:00 pm	33.25±0.45	65.32 ±0.47
8:00 pm	31.62±0.82	64.43 ±0.78
12:00 am	29.85±0.91	60.67 ±0.82
04:00am	28.87±0.43	55.22 ±0.11

Statistical analysis

Data was statistically analyzed with one way analysis of variance using statistical package SAS (SAS Institute, 1992). Least significant test was used to compare the differences among treatment means to find the significant difference. P value less than 0.05 was statistically considered significant.

RESULTS

Mean serum antibody titer against ND of four different broiler strains reared in TN and HAT zone is shown in Table IV. No significant difference was found in mean serum antibody titer of four different broiler strains against ND in TN zone, while in HAT zone, significantly higher mean serum antibody titer against ND was found in Ross and Arbor Acres followed by Cobb and Hubbard at all recorded stages.

Mean serum MDA of four different broiler strains reared in TN and HAT zone are presented in Table V. Significantly (P<0.05) lower serum MDA was recorded in all broiler strains in TN zone at all recorded stages. In HAT zone, significantly lower (P< 0.05) serum MDA was recorded in Ross and Arbor Acres at all recorded stages except day 21. On day 21, no significant difference was recorded in mean serum MDA level of different broiler strains in HAT zone.

Table IV.- Antibody titer against Newcastle disease (ND) of different broiler strains reared under thermo neutral (TN) and high ambient temperature (HAT) zones.

Zone	Strain	Day 21	Day 28	Day 35	Day 42
TN zone	Ross	1647.8±4.21^{a}	1561.5±6.23^{a}	1406± 4.76^{a}	1253.3± 3.35^{a}
	Hubbard	1658.0±8.20^{a}	1557.5±12.3^{a}	1433.8± 8.11^{a}	1255.8± 11.75^{a}
	Cobb	1647.8±3.90^{a}	1552±10.15^{a}	1409±7.59^{a}	1266.3±7.50^{a}
	Arbor Acres	1645.5±4.34^{a}	1532.5±5.2^{a}	1407.2±3.81^{a}	1253.3±5.45^{a}
HAT zone	Ross	1594.5±6.23^{b}	1480±4.56^{b}	1348±7.78^{bc}	1207.5±3.77^{b}
	Hubbard	1553.8±8.25^{c}	1432±10.68^{c}	1327.5±3.22^{bc}	1172.3±4.20^{c}
	Cobb	1544.5±11.44^{c}	1440±15.54^{c}	1320±7.07^{c}	1169±17.96^{c}
	Arbor Acres	1586.8±4.26^{b}	1474±1.29^{b}	1353.8±5.89^{b}	1215±10.40^{b}
P-values		0.001	0.0071	0.032	0.016

Means in the same column with different superscripts are significantly different (P<0.05).

Table V.- Serum malondialdehyde (MDA) (nmol/ml) (Mean±SD) of different broiler strains reared under thermo neutral and high ambient temperature.

Zone	Strain	Day 21	Day 28	Day 35	Day 42
TN zone	Ross	6.90±0.14^{b}	9.08±0.06^{c}	9.41±0.23^{c}	11.69±0.09^{c}
	Hubbard	6.76±0.13^{b}	8.94±0.07^{c}	8.93±0.28^{c}	11.30±0.21^{c}
	Cobb	6.45±0.28^{b}	8.87±0.05^{c}	8.8±0.32^{c}	11.37±0.17^{c}
	Arbor acres	6.60±0.21^{b}	8.98±0.031^{c}	9.14±0.28^{c}	11.72±0.20^{c}
HAT zone	Ross	8.45±0.17^{a}	10.26±0.08^{b}	10.46±0.24^{b}	13.22±0.28^{b}
	Hubbard	9.18±0.14^{a}	11.65±0.12^{a}	11.8±0.48^{a}	14.34±0.07^{a}
	Cobb	9.22±0.26^{a}	11.79±0.12^{a}	11.88±0.41^{a}	14.32±0.41^{a}
	Arbor Acres	8.47± 0.18^{a}	10.15± 0.08^{b}	10.37± 0.19^{b}	13.47±0.22^{b}
P-value		0.026	0.0001	0.0043	0.0003

Means in the same column with different superscripts are significantly different (P<0.05).

Table VI.- Serum paraoxonase (U/L) (Mean±SD) of different broiler strains reared in thermo neutral and high ambient temperature zones.

Zone	Strain	Day 21	Day 28	Day 35	Day 42
TN zone	Ross	13.36±0.20 [a]	12.75±0.035 [a]	12.07±0.097 [a]	11.54±0.281 [a]
	Hubbard	13.64±0.153 [a]	12.82±0.26 [a]	12.26±0.090 [a]	11.89±0.167 [a]
	Cobb	13.48±0.20 [a]	13.01±0.13 [a]	12.05±0.10 [a]	11.79±0.068 [a]
	Arber Acres	13.35±0.269 [a]	12.77±0.076 [a]	11.98±0.134 [a]	11.57±0.086 [a]
HAT zone	Ross	12.30±0.20 [b]	12.19±0.29 [b]	11.41±0.099 [b]	10.86±0.087 [b]
	Hubbard	11.72±0.17 [c]	11.52±0.11 [c]	10.76±0.092 [d]	10.82±0.118 [b]
	Cobb	11.42±0.075 [c]	11.32±0.060 [c]	10.94±0.092 [cd]	10.50±0.106 [b]
	Arber Acres	12.58±0.134 [b]	12.16±0.080 [b]	11.20 ±0.076 [bc]	10.82±0.114 [b]
P-value		0.0038	0.0035	0.0014	0.029

Means in the same column with different superscripts are significantly different at $\alpha = 0.05$.

Mean serum PON1 activity of four different broiler strains reared in TN zone and HAT zone are presented in Table VI. Significantly ($P<0.05$) higher serum PON1 was recorded in all broiler strains in TN zone at all recorded stages. In HAT zone, significantly higher ($P< 0.05$) serum PON1 was recorded in Ross and Arber Acres at all recorded stages except at day 42. On day 42, no significant difference was found in mean serum PON1 of all broiler strains.

DISCUSSION

Heat stress has been known for significant reduction in the growth performance and health status of the birds by reducing the feed intake and production of free radicals. These changes in birds increase the incidence of diseases in heat stressed birds. The results of the present research showed that HAT zone significantly affected mean serum antibody titer against ND. Heat stress damages the immune system of the birds in several ways. Heat stress reduces the formation of immune cells (T and B cells), suppresses the phagocytic activities, lowers the white blood cells (WBCs) and heterophils to lymphocyte ratio (Khan *et al*., 2012a). In addition, heat stress also impairs the humoral immunity and synthesis of antibodies and increases the inflammatory cytokines.

Heat stress causes oxidative damage and reduces the weight of lymphoid organs (Chand *et al*., 2014) which may be the probable cause of reduced antibody production against ND disease. It has been reported that stressful condition increases the activity of adrenal gland which increases the production of serum corticosteroids (Ihsanullah *et al*., 2017) causes suppression of cell proliferation. The decreased antibody titer at high ambient temperature may also be due to the increased inflammatory cytokines and depression of T-helper cells. T-helper cells are responsible for production of antibodies (Khan *et al*., 2012b). Exposing broiler chicks to high ambient temperature impairs the functions of immune system in hen (Chand *et al*., 2016), suppresses the defense mechanism of birds and hence decreases antibody production. Several research reports have documented the negative effects of heat stress on the antibody titre in chickens (Khan *et al*., 2012b; Chand *et al*., 2014).

Temperature zone significantly affected serum MDA, and PON1 of different broiler strains at all recorded stages. The MDA is a very important byproduct of lipid peroxidation of the lipids contents of the cell membrane and directly responsible for the damage of the cell. High ambient temperature zone significantly increased serum MDA and decreased PON1 of different broiler strains. High ambient temperature increases lipid peroxidation as a result of increased ROS which causes damage to the cellular protein (Chand *et al*., 2016). Exposure of broiler chicks to high ambient temperature increases lipid peroxidation which is indicated by the increased MDA concentration (Chand *et al*., 2016). The PON1 is a reliable indicator of antioxidant system and its concentration is generally decreased during the stress condition. This enzyme is also very important in the prevention of the oxidation of the high density lipoprotein. Our findings regarding lower PON1 under high ambient temperature are in agreement to the results of Chand *et al*. (2016).

CONCLUSION

The results of the present study revealed that immune response and antioxidant status were significantly affected by HAT and strains of birds. At higher temperature, the measured parameters were most affected in Hubbard

and Cobb strains. Ross and Arbor Acres showed better resistance to change in the studied physiological parameters during exposure to high ambient temperature. It is concluded from results that Cobb and Hubbard strains exhibited more susceptibility to heat stress, while Ross and Arbor Acres strains were more tolerant to summer high ambient temperature of tropical areas of Peshawar.

Statement of conflict of interest

Authors have declared no conflict of interest.

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