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Pattern of Leptin Secretion and Oxidative Markers in Heat-Stressed Pigeons

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Abstract: The effect of heat stress on oxidative stress was studied in pigeons. Pigeons were either kept at thermo neutral room at 22°C, 24 hours/day or heat stress room at 34°C for 8 hours/day followed by 22°C for the rest of the day during the 40 days experiment. Increased heterophils: lymphocyte ratio was evident indicating stress in pigeons. Parallel increase in leptin, metabolites and oxidative markers was observed suggesting a modulating role of leptin during oxidative stress in pigeons.

Key words: Leptin, pigeons, heat stress, oxidative markers

INTRODUCTION

Heat stress in birds is accompanied by a decrease in feed intake, basal metabolic rate and immunity (Bobek *et al.*, 1996; Bartlett and Smith, 2003). Growth rate, hatchability, mortality are also adversely affected (Rozenboim *et al.*, 2007). When the temperature and relative humidity exceed the comfort level of a bird, it loses the ability to dissipate heat. This leads to physiological changes including a reduction in feed intake for reducing metabolic heat production (Teeter *et al.*, 1985; Geraert *et al.*, 1996). Humoral and neural pathways have been identified that link appetite and energy balance.

Leptin has been shown to play an important role in the regulation of food intake, energy expenditure and metabolism (Friedman and Halaas, 1998). Leptin has been identified in many organs in birds (Taouis *et al.*, 1998; Ashwell *et al.*, 1999). The effect of leptin on reducing feed intake in chickens has been demonstrated by intraperitoneal injection of recombinant chicken leptin (Dridi *et al.*, 2000). This study was carried out to determine pattern of leptin secretion among other oxidative markers in pigeon subjected to heat stress.

MATERIALS AND METHODS

Bird care and treatment: Fifty six 2 week old pigeons from a commercial company were used in the study. The birds were assigned according to their initial weight to two treatment groups consisting of 28 birds each:

Group 1 birds (Thermo neutral Group) were fed the basal diet and kept in temperature-controlled rooms at 22°C, 24 hours per day for 40 days.

Group 2 birds (Heat-stress Group) were fed the basal diet and kept at 34° C for 8 hours per day from 9.00 to 17.00 followed by 22° C for 16 hours.

Collection of samples: At the end of 40 days blood samples were collected from birds for hematology. The rest of blood was centrifuged at 3000 g for 10 min and plasma was separated and stored at -20°C until analysis.

Laboratory analysis: The differential count of heterophils and lymphocytes (100 cells/field) was carried out by light microscope using an immersion objective for blood smear stained Rosenfeld, and results were expressed. Serum leptin was determined using RIA Kit (Multi species leptin RIA Jit, Linco Research, St Charles, MO) according to the manufacturer recommendations (Al-Azraqi 2007). The limit of sensitivity was 0.5 ng/ml. The intra- and inter-assay coefficient of variation were 4.3% (n=20) and 5% (n=15) , respectively.

Lipid peroxidation was assessed by estimation of free malondialdehyde (MDA) spectrophotometrically. This assay is based on the reaction of N-methy-2-phynylindole with MDA, producing a carbocyanine dye whose absorbance can be read at 586 nm (Judge *et al.*, 2008).

Protein oxidation was assessed by estimation of protein carbonyls using an enzyme immunoassay kit (Zenith Technology, Dunedin, New Zealand). The coefficient of variation for protein carbonyls and MDA was 3 % and 4%, respectively.

Statistical methods: The data was analyzed using GLM procedure of SAS software. Significant differences among the means was range measured using Multiple range test at $p < 0.05$

RESULTS AND DISCUSSION

Effect of heat stress on plasma concentration of leptin, oxidative markers, heterophils and lymphocyte in pigeons are summarized in Table 1 and illustrated in Fig. 1a,b,c.

Heat stress significantly ($p < 0.05$) increased MDA,

Table 1: Effect of ambient temperature regimens on mean (± SD) plasma concentration of leptin, metabolites, oxidative markers, neutrophils and lymphocytes of pigeon

Parameter	Group 1 (Thermo neutral Group)	Group 2 (Heat stress Group)
Glucose (mg/dl)	245 ± 5.3	301 ± 11.1*
Cholesterol (mg/dl)	162 ± 6.1	200 ± 10.2*
Triglyceride (mg/dl)	160 ± 10.2	211 ± 12.1*
Leptin (ng/ml)	1.13 ± 0.26	2.1 ± 0.33*
MDA** (µmol/L)	0.82 ± 0.03	2.41 ± 0.12*
Protein carbonyls (nmol/L)	0.91 ± 0.04	1.81 ± 0.11*
Heterophils (%)	44 ± 2	51 ± 2*
Lymphocytes (%)	56 ± 2	49 ± 2*

*p < 0.05; significant difference compared to values in Group 1.

protein carbonyls, glucose, cholesterol and leptin concentrations in heat stress group compared to thermo neutral control group. Heat stress also significantly ($p < 0.05$) increased heterophils percentage and decreased lymphocytes percentage in the heat stress group compared to the thermo neutral group. The ratio of heterophils: lymphocytes, which was 0.7 in the control group, increased to 1.16 in the heat stress group.

In this study, heat stress in pigeons was first assessed by heterophils: lymphocytes ratio that has been shown to be increased significantly. Heterophils: lymphocytes ratio has been indicated to be a good quantitative measure of stress (Zulkifli and Siegel, 1995; Borges *et al*., 2003).

The present study shows that metabolites such as glucose, cholesterol and triglycerides concentrations have been increased in heat-stressed pigeons. Similar findings on effect of heat stress on concentrations of glucose and cholesterol (Sahin *et al*., 2004) and triglycerides (Eid *et al*., 2003) have been reported in birds. Stressors such as high ambient temperature induce secretion of corticosteroids in birds (Siegel, 1995), which consequently exert catabolic effects. Thus, resulting in muscle wasting and decreased growth (Odedra *et al*., 1983; Hayashi *et al*., 1994).

The oxidative stress induced by heat stress was reflected in the increase of protein oxidation (increased protein carbonyls) and lipid peroxidation (increased MDA) (Silvestro *et al*., 2002; Turton *et al*., 2002). Heat stress (Sahin *et al*., 2004), exercise (Silvestro *et al*., 2002) and ischemia (Judge *et al*., 2008) can result in increased protein oxidation and lipid peroxidation.

Parallel increase in the concentrations of leptin and metabolites was demonstrated in this study. Leptin directly affects glucose and lipid metabolism in isolated adipocytes, myotubes and skeletal muscles (Muoio *et al*., 1997; Ceddia *et al*., 1998). Leptin causes reduction in adipose tissue mass and increases free fatty acids oxidation in peripheral tissue (Muoio *et al*., 1997; Shimabukuro *et al*., 1997). Moreover, in pregnant obese

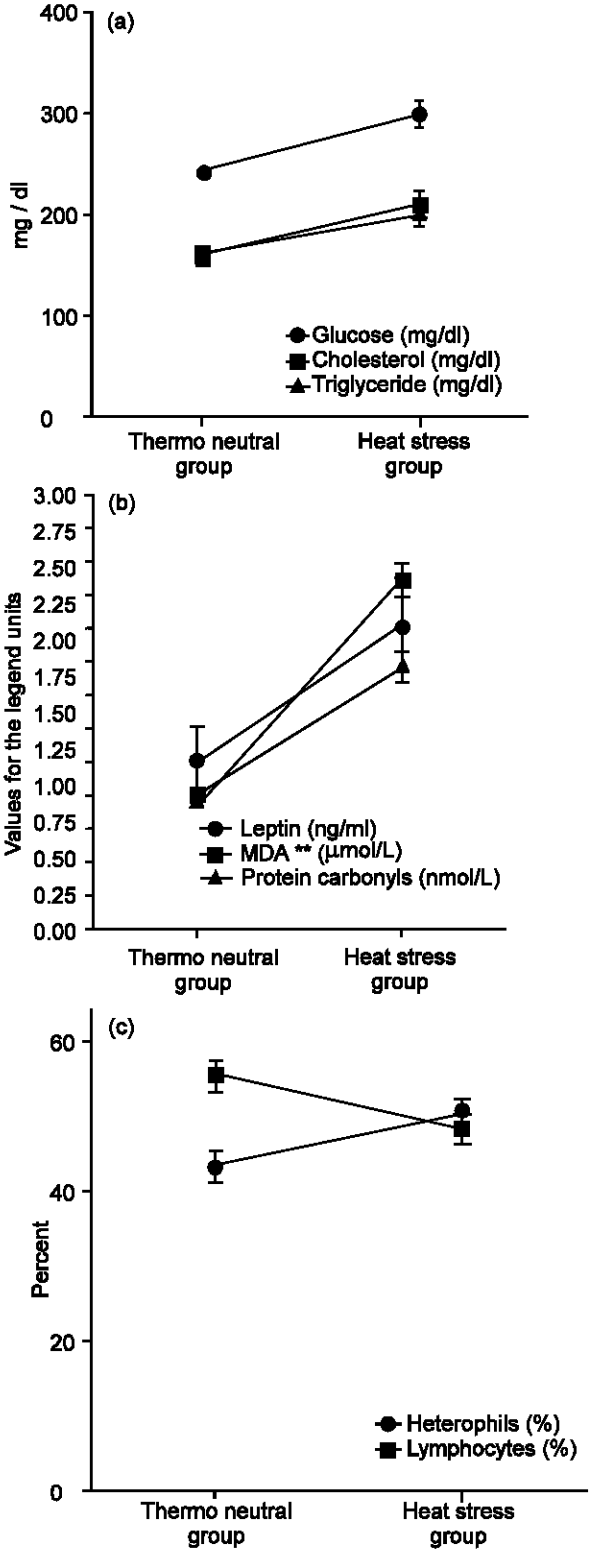

Fig. 1: Illustrating the differences between the thermo neutral group and the heat stress group in the parameters of the study.
** MDA is the abbreviation for malondialdehyde.

women, increased fat oxidation has been correlated with increasing leptin concentration (Okereke *et al.,* 2004). According to the present results, via its ability in controlling lipogenesis (Cassy *et al.,* 2003), leptin seems to partially modulate the oxidative stress in pigeons.

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