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Effect of Early-Age Acclimation on Some Physiological, Immunological Responses and Chromosomal Aberrations in Muscovy Ducks During Exposure to Heat Stress

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Abstract: A total of 150 one day old Muscovy ducklings, were used in the present study to assess the effect of early heat acclimation on some plasma parameters, immune responses and chromosomal aberration during heat stress. Ducks were divided into three equal groups. The control group, was maintained under 32-35°C during the first week, meanwhile, the second and third groups were exposed to high temperature 38°C for 24 h at day 3of age and at day 5 of age, respectively. All the groups were exposed to high temperature (42-43°C) for 4hr for three consecutive days at 9 weeks of age. Chromosomal aberrations were determined at seven days and 9 weeks of age during exposure to heat stress. Early heat acclimation of 3 days had increase in plasma levels of total proteins and uric acid compared to the other groups during exposure to heat stress. While, early heat acclimation caused a significant ($P<0.05$) reduction in plasma lactate dehydrogenase (LDH) activity as compared to the control group during exposure to heat stress. The relative weights of spleen, thymus and bursa were significantly ($P<0.05$) higher in early heat acclimated (3 days) group as compared to the other groups during heat stress. There was a significant ($P<0.05$) improvement in Heterophil / Lymphocyte (H/L) ratio sticking and cell mediated immune (CMI) response to phytohemagglutinin-p (PHA-P) with early heat acclimation. Acclimated ducklings had lower significant($P<0.05$) differences in all types of chromosomes aberration compared with the control group during exposure to heat stress at seven days of age but, differences were not significant in all types of aberration among all groups during heat stress at 9 weeks of age. In conclusion, the present data indicated that early heat acclimation especially at 3 days of age had some beneficial effects on some physiological and immunological responses; and chromosomes aberrations in Muscovy ducks during exposure to heat stress.

Key word: Muscovy ducks · Heat acclimation · Physiological · Immunological genetics · Responses

INTRODUCTION

In hot climate regions declined poultry production is generally found and is attributed to high ambient temperature, especially when coupled with high humidity which imposes severe thermal stress on poultry. It was found that Pekin, Rouen and Khaki Campbell ducks had light body weight in Egypt due to the unfavorable environmental conditions including high air temperature [1]. A temperature of 23-25ºC has been found to maximize growth of 1-7 weeks old duckling and the performance of ducks decreased with the increase in environmental temperature above 29°C [2,3]. Also, Shafie *et al*. [4] reported that ducks are affected at a lower threshold environmental temperature 23°C as compared to chickens (20°C) and this indicates that the thermoneutrality zone of ducks is lower that of chickens. Heat stress in birds leads to many biochemical and physiological changes in body such as, hyperthermia [5,6] as well as decreased plasma protein [6], Plasma protein response may be a useful evaluation criterion, whereas, evidence suggests that plasma protein may give an indication of the effect of heat stress in chickens [7,8].

Environmental stress and physiological response has been well documented as a cause of humoral and cell-mediated immunsuppression in avian species, initiated by hypothalamus-pituitary-adrenal cortical pathway [9]. The Heterophil / Lymphocyte (H/L) ratio is now established as a widely accepted indicator for determining stress in ducks [10]. Also, the relative

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lymphoid organs weight is considered as an indication of the immunological advances [11]. Although limited information is available on the effect of high temperature on cell mediated immune response. A depression in the phagocytic potential of chicken macrophages during *in vitro* heat stress conditions was reported [12].

Phytohemagglutini-p (PHA-P) is a lectin isolated from red kidney bean and stimulates T-cell proliferation with minimal on B cells. It is considered as good *in vivo* measures of T-lymphocyte function [13].

There is an apparent paucity of information on effect of heat stress on cytogenetic, especially in avian. Exposure of cells or organisms to thermal stress is known to result in alteration in the integrity of the nucleolus besides it adversely affecting the structure and function of the centrosome [14]. Asanami and Shimono [15] suggested that body temperatures of 39.5°C or higher for more than 30 minute induce micronuclei in bone marrow cells.

Many attempts have been made to alleviate the deleterious effects of thermal stress on birds. Acclimation o f birds to heat was achieved by exposing birds to cyclic temperature [16,17] or neonatal exposure (early heat acclimation) to heat stress and this resulted in a greater heat resistance [18,19]. The technique of temperature conditioning takes advantage of the immaturity of the mechanism of temperature regulation in young birds during their 1st wk of life [20] that involves sympathetic neural activity and the integration of thermal information in the hypothalamus [21], the potential thermotolerance can thus be incorporated into the developing mechanisms of thermoregulation.

The aim of this study was to ameliorate the deleterious effect of heat stress by early heat acclimation and to detect the physiological and immunological changes; and chromosomes aberrations following heat stress.

MATERIAL AND METHODS

The experiment was carried out in the Research Station of Waterfowls at El-Serw, Domiat governorate, which belongs to the Animal Production Research Institute, Ministry of Agriculture, Egypt. Some physiological, immunological responses and chromosomal aberrations in Muscovy ducks were monitored after early age acclimation.

Birds, Diet and Treatments: A total of 150 Muscovy ducklings on day one, was used in this study. At the day of hatch, all ducks were randomly divided into three equal groups. The control group, was maintained under 32-35°C during the first week, as a recommended thermal conditions and then gradually decreased (0.5°C per day) until 23 ± 1°C was reached by day 21. The second and third groups were exposed to high temperature 38°C for 24h at day 3 of age (early acclimation 3) and at day 5 of age (early acclimation 5), respectively [22] whereas the temperature was controlled by gas heater with thermostat. All groups were reared under the natural environmental temperature then exposed to high temperature (42-43°C) for 4hr at for three consecutive days at 9 weeks of age. Water and diets were given *ad libitum* throughout the rearing period. Crude protein and metabolizable energy levels in the starter and growing diets were 20% crude protein and 2900 kcal/diet and 15.5% crude protein and 2700 kcal/diet, respectively. All diets met the nutrient requirements of Muscovy ducklings [23].

Plasma Parameters: At 9 weeks of age, three birds from each group were randomly chosen. Blood samples were collected from wing vein into dry clean centrifuge tubes containing drops of heparin and centrifuged for 15 minutes at 3000 rpm. The plasma samples were stored in the deep freezer at approximately - 20°C till the time of chemical analysis. Plasma samples were analyzed for total protein [24], uric acid [25] and lactate dehydrogenasee [26] values using chemical kits.

Measurements of Immunocompetence

Hematological Parameters: White blood cells, lymphocytes and heterophils were counted in fresh blood samples using hemocytometer and light microscope at 9 weeks of age during exposure to heat stress[add reference for counting].

Lymphoid Organs and Heart Weights: At 9 weeks of age during exposure to heat stress three ducks of each groups were weighed and slaughtered then the weights of lymphoid organs (spleen, thymus gland and Bursa of Fabricious) and heart were recorded to the nearest 0.1g. The weights of these organs were calculated relative to body weight.

Phytohemagglutinin Injection (*In Vivo* Cell-Mediated Immunity Assay): Response induced *in vivo*` by mitogen was evaluated by injection of phytohemagglutinin-P

(PHA-P) according to Sanders *et al*. [27]. Five ducks from each groups at 9 weeks of age were used, each duck was intradermally injected in the toe web of the left foot between the second and the third digits of ducks with 100 µg phytohemagglutinin-P (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml of sterile saline solution. Toe web was measured with a micrometer before injection and at 24, 48 and 72 hr after PHA-P injection. The toe web swelling was calculated as the difference between the thickness of the toe web before and after injection.

Chromosomal Aberrations: At day seven and 9 weeks of age, the chromosomal aberrations traits were studied during heat stress in five birds from each treatment. All ducks groups were injected ip with colchicines 3 h before killing. Bone marrow cells were prepared according to Yosida and Amano [28]. Slides were stained with phosphate buffered giemsa. 50 metaphases were studied from each somatic cell for each duck. Structure and numerical aberration were recorded.

Statistical Analysis: Data were analyzed by the least squares analysis of variance using the General Linear Models procedure of the statistical analysis model [29]. The statistical model was as follows:

$$Yi = \mu + Ti + ei$$

Where:

Yi = Observation of the duck;

µ = Overall mean, common element to all observations;

Ti = Effect of the early acclimation treatment (i = 1, 2, 3); and ei = Random error component assumed to be normally distributed. Data estimated in percentage were transformed with the arcsine square-root procedure to normalize variance before analysis and were retransformed again to the original scale before presentation. The differences among means were tested using Duncan's New Multiple Range Test [3].

RESULTS

Plasma Parameters: Early heat acclimation (3 days) caused a significant (P=0.05) increase in plasma total proteins as compared to the other groups during exposure to heat stress (Table 1).

Table 1 shows that early heat acclimation (3 days) caused a significant (P=0.05) increase in plasma uric acid as compared to the other groups during exposure to heat stress. Moreover the results in table 1 showed that early heat acclimation caused a significant (P=0.05) reduction in plasma lactate dehydrogenase activity as compared to the control group during exposure to heat stress.

Lymphoid Organs and Heart Weights: Table 2 shows the effect of early heat acclimation on some relative weight of lymphoid organs and heart. The relative weights of spleen, thymus and bursa were significantly (P<0.05) higher in early heat acclimated (3 days) group as compared to the other groups during exposure to heat stress, but there were no significant differences between early heat acclimated (5 days) and control groups during exposure to heat stress in previous traits. Early heat acclimation caused a significant (P=0.05) reduction in the relative weight of heart as compared to the control group during exposure to heat stress (Table, 2).

Results in Table 3 indicated that, early heat acclimation (3 days) caused a significant (P=0.05) increase in white blood cells as compared to the other groups during exposure to heat stress. Lymphocytes were insignificantly decreased in early heat acclimated group as compared to control group during exposure to heat stress. Also, heterophils were significantly decreased in early heat acclimated group as compared to the control group during exposure to heat stress. H/L ratio was significantly (P=0.05) increased after exposure of heat stress.

Table 1: Effect of Early Heat Acclimation on Plasma Parameters in Muscovy Ducks during Heat Stress at 9 Weeks of Age (Mean ±SE)

	Treatment		
Items	Control	Early acclimation3	Early acclimation 5
Total Protein g/dl	$3.88^{b} \pm 0.4$	$6.00^{a} \pm 0.\ 0.58$	$4.19^{b} \pm 0.37$
Uric acid mg/dl	$4.38^{b} \pm 0.51$	$5.69^{a} \pm 0.51$	$3.94^{b} \pm 0.17$
Lactatedehydrogenase(LDH,U/L)	$596.61^{a} \pm 1.9$	$449.87^{c} \pm 0.98$	$526.46^{b} \pm 1.11$

[a, b] Means with different superscripts in the same row differ significantly (P=0.05)

Table 2: Effect of Early Heat Acclimation on Relative Weights of Lymphoid Organs and Heart in Muscovy Ducks during Heat Stress at 9 Weeks of Age (Mean ±SE).

	Treatment		
Items	Control	Early acclimation 3	Early acclimation 5
Spleen weight, %	76.02^{b}±1.41	101.41^{a}±0.53	80.09^{b}±1.34
Thymus weight, %	233.86^{b}±0.68	281.08^{a}±0.79	277.52^{a}±0.78
Bursa weight, %	82.83^{b}±0.4	119.70^{a}±0.69	85.42^{b}±0.40
Heart weight, %	0.77^{a}±0.03	0.64^{b}±0.02	0.64^{b}±0.02

$^{a, b}$ Means with different superscripts in the same row differ significantly (P=0.05)

Table 3: Effect of Early Heat Acclimation On Hematological Parameters In Muscovy Ducks During Heat Stress At 9 Weeks Of Age(Mean ±SE)

	Treatment		
Items	Control	Early acclimation 3	Early acclimation 5
WBC's (10^3/mm^3)	26.55^{b}±0.4	29.17^{a}±0.43	27.51^{b}±0.42
Lymphocytes (L,10^3/mm^3)	11.76^{a}±0.4	10.95^{a}±0.35	11.61^{a}±0.40
Heterophils (H,10^3/ mm^3)	5.25^{a}±0.40	4.49^{b}±0.4	4.95^{b}±1.04
H/L ratio	0.44^{a}±0.01	0.41^{c}±0.01	0.42^{b}±0.01

$^{a, b}$ Means with different superscripts in the same row differ significantly (P=0.05)

Table 4: Effect of Early Heat Acclimation on Cell Mediated Immunity in Muscovy Ducks during Heat Stress at 9 Weeks of Age(Mean ±SE).

	Treatment		
Items	Control	Early acclimation 3	Early acclimation 5
Before injection	0.153^{a}±0.02	0.150 ^{a}±0.01	0.140 ^{b}±0.01
24 hr-post injection	0.283 ^{a}±0.03	0.251^{b}±0.02	0.267 ^{b}±0.01
48 hr-post injection	0.258 ^{a}±0.02	0.248 ^{a}±0.01	0.250 ^{a}±0.01
72 hr-post injection	0.231 ^{a}±0.01	0.203 ^{a}±0.01	0.212 ^{a}±0.01

$^{a, b}$ Means with different superscripts in the same row differ significantly (P=0.05)

Table 5: Effect of Early Heat Acclimation on Chromosomal Aberration in Bone Marrow Cells of Muscovy Ducks during Heat Stress at Day Seven Age (Mean ±SE).

	Treatment		
Items	Control	Early acclimation 3	Early acclimation 5
Break	11.0^{a}±0.28	1.8^{c}±0.51	3.4^{b}±0.52
Polypolid	14.8^{a}±0.49	2.4^{c}±0.45	6.0^{b}±0.77
Stickness	17.0^{a}±0.66	2.0^{c}±0.32	8.4^{b}±0.50
Pulverization	13.2^{a}±0.33	1.4^{c}±0.4	6.2^{b}±1.01
Total	56.0^{a}±1.65	7.6^{c}±0.85	24.0^{b}±1.65

$^{a, b}$ Means with different superscripts in the same row differ significantly (P=0.05)

Table 6: Effect of Early Heat Acclimation on Chromosomal Aberration in Bone Marrow Cells of Muscovy Ducks during Heat Stress at 9 Weeks of Age (Mean ±SE).

	Treatment		
Items	Control	Early acclimation 3	Early acclimation 5
Break	1.40^{a}±0.58	0.60^{a}±0.54	0.60^{a}±0.54
Polypolid	1.60^{a}±0.2	1.00^{ab}±0.36	0.40^{b}±0.32
Stickness	1.40^{a}±0.58	0.80^{a}±0.38	1.00^{a}±0.36
Pulverization	1.00^{a}±0.36	0.40^{a}±0.32	0.60^{a}±0.32
Total	5.40^{a}±0.38	2.80^{b}±0.49	2.60^{b}±1.05

$^{a, b}$ Means with different superscripts in the same row differ significantly (P=0.05)

Cell-mediated Immunity (CMI): Cell-mediated immunity response was significantly (P=0.05) lower in early heat acclimated groups at 24h after PHA-P injection as compared to the control group during exposure to heat stress (Table, 2). While, CMI response were insignificantly lower in early heat acclimated groups at 48 and 27h after PHA-P injection as compared to the control group during exposure to heat stress (Table 4).

Chromosomal Aberrations: Results of cytogenetic analysis presented in Tables 5 and 6 and Figures 1 and 2 show different types of chromosomal aberrations which include break, polyploidy, stickiness and pluvirazation at day seven and 9 weeks of age. Results in table 5 reveal that acclimated ducklings had lower significant (P=0.05) differences in all types of aberration compared with the control group during exposure to heat stress at seven days of age.

Results in Table 6 revealed that there were no significant differences in all types of aberration among all groups during heat stress at 9 weeks of age. Beside that, the present results revealed that there was a significant (P<0.05) improvement in chromosome aberration test with early heat acclimation in old ducks (9 weeks).

DISCUSSION

In the present study, exposure to heat stress in Muscovy ducks leads to many physiological, immunological responses and chromosomal aberrations. Plasma showed a significant increase in total proteins in early heat acclimation. Similar findings were obtained in broiler chickens during high temperature exposure [31]. Total plasma proteins may be used as useful criterion for heat stress in birds [8]. However, an opposite trend in chickens was recorded [32]. Differences among investigations may be due to differences in poultry species and/or duration of heat stress exposure.

In this study increased in plasma uric acid during exposure to heat stress was evident. In this respect, it was found that kidney function improved in heat-acclimated broilers than non acclimated broilers [33]. Also, this finding may be due to a decrease of protein consumption in heat stress group rather than affecting kidney function. Also, Geraert *et al.* [34] suggested that high plasma uric acid concentration in heat – exposed chickens (32°C) compared with non exposed group (22°C) is due to the increase of protein catabolism.

The reduction in plasma LDH, probably begin to rise at a time when a take disturbance of the cardiovascular system due to heat stress. Plasma LDH activity was reduced in early heat acclimated groups and may be due to that birds previously experiencing a high temperature showed a lower standing-lying frequency during subsequent exposure to high temperature and this may indicate that experienced birds are less nervous during subsequent exposure to high temperature [35].

The differences in the weight of lymphoid organs and heart weights may be a result of reduction in feed in take, there by providing fewer nutrients for the proper development of those organs [36].

The mechanisms associated with the induction of thermo tolerance by early-age temperature conditioning may be due to haemodynamic changes [19, 37]. Also, our results are in agreement with Atta [38] and Maxwell and Robertson [39] who found that heat stress caused suppression in the activity of T and B- lymphocytes and macrophages. On the other hand, heterophils increase and lymphocytes decrease when birds are stressed, so that ratio between them is a good index of response to a stressor [9, 40]. The changes in lymphocyte and heterophil percentage due to the release of ACTH through the hypothalamus pituitary axis after exposing the birds to stress which in turn decrease the lymphocyte percentage, while heterophil percentage increase [40]. The changes in lymphocyte and heterophil percentage are attributed to the redistribution of cell out of the circulation and into secondary lymphoid organs [41]. This interpretation would be consistent with the finding of Mashaly *et al*. [42] who attributed the decrease of ACTH to redistributed of T-lymphocyte only (not B-lymphocyte) subpopulation between the peripheral blood and spleen. There was a significant improvement in H/L ratio sticking with early heat acclimation. This confirmed the hypothesis that early heat acclimation enhanced the ability to heat resistance.

In this study, the cell-mediated immunity response was significant high in heat stress [36,43]. The immunological function of thymus is to provide a specific environmental essential for T-cells differentiation, which essential for cell mediated immunity and modulation of immune response [44]. He added that the differentiation is through subpopulation of thymic cells including T-helper, T-cytotoxic and T-supperssor cells. From this point of view, decreased in the relative weight of thymus by heat stress in the present study may be lead to decrease in cell mediated immunity.

Overall, the present data suggest that used of early heat acclimation may reflect the positive increase in the immunity through decreasing the CMI.

Asanami and Shimono[15] and Asanami *et al*. [45] observed positive responses in mouse exposed for 6h to t 40 and 41°C for 24h and 42°C in the chromosome aberration test, respectively. In the micronucleus test, they observed positive responses at 31, 33 and 40°C for 24h and 42°C for 2h. Results suggested that in Chinese hamster cells line, hypothermic conditions can induce micronuclei while hyperthermic conditions can induce both chromosome aberration and micronuclei. From the present results, it was observed that chromosomal aberrations of ducklings were more affected by heat stress at early age. It was suggested that the potential thermotolerance can not be incorporated into the developing mechanisms of thermoregulation especially in control group. Whereas, the development of neuronal hypothalamic thermosensitivity can induce between day 28 of incubation and the 10th day after hatching in Muscovy duckling [46]. Moreover, Laszlo [47] suggested that there is a relationship between thermotolerrance and heat shock proteins (hsp) synthesis rate. Furthermore, it has been explained that heat shock proteins bind to chromosomes following heat stress and they take part in chromosome condensation and on recovery induce damages like chromatid stickiness. Mamon and Kutskova [48] added that high temperature has a role in inducing damages of mitotic chromosomes in *Drosophila melanogaster*. So, it was suggested that old ducks were more resistance to heat stress t due to potential thermotolerance can incorporat into the developing mechanisms of thermoregulation [21].

In conclusion, exposing ducks to high ambient temperature leads to negative effects on some physiological and immunological responses and cytogenetic effects. It has been suggested that early heat acclimation can be used to improve these adverse effects. So, early heat exposure method is recommended for more adaptive ducks during hot conditions.

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