BLOOD BIOCHEMICAL AND IMMUNOLOGICAL RESPONSES OF MUSCOVY DUCKLINGS TO EARLY AGE THERMAL MANIPULATION

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SUMMARY

The main objective of this study was to investigate the beneficial effects of early age epigenetic adaptation of newly hatched ducks on their post hatch growth performance, physiological and blood biochemical changes, along with oxidative stress and immune responses. Total of 200, day old ducklings were brooded at 33 ±1°C for the first three days post hatching, and then were divided into two equal groups (100 chicks each), a control group maintained under the brooding temperature, while the other group was exposed to early age thermal conditioning at 39.5 ±1°C for 4 h at the 4th, 5th and 6th days post hatching. After that both groups were subjected to 30 ±1°C from the 7th to the 10th day of age then reduced gradually to reach 24 ±1°C by day 28 of age. From the beginning of the 5th week of age until the end of the experiment (12 weeks), all birds have similar feeding and managerial conditions. Blood samples were collected after 24 hours of the first exposure time, and again after the last exposure (at 80 days of age). Results showed that LBW and BWG of ducks were significantly ($P \leq 0.05$) higher for those exposed to TM compared by those of the control (TN) treatment. Feed consumption was not significantly affected by early age TM; however, the cumulative FC was significantly increased for the heat-exposed ducks. Meanwhile, feed conversion ratio (FCR) was significantly better for the TM- exposed ducks than those of TN ones. Body temperature and respiration rate were significantly decreased at the latter stage of growing period (12 week) by early-age thermal conditioning. Plasma lysozyme activity of Muscovy ducks was insignificantly increased in early heat exposed group compared to the control one. Red blood cells count, hemoglobin level and PCV were significantly increased in TM exposed ducks compared to the TN ones. Plasma thyroid hormones (T3, T4) and corticosterone levels were significantly increased at the end of experiment as a result of thermal conditioning. A similar trend was also observed for the H:L ratio which was the highest in TN birds. Corticosterone and Heterophils: lymphocytes ratio decreased in the thermally manipulated-duckling compared to control group. Plasma MDA was significantly decreased in early heat exposed group compared to the control group. Superoxide dismutase and catalase enzymes increased in TM exposed ducks compared to the control one. It is concluded that a 3 days of early age thermal conditioning could be used as a practical approach to alleviate the negative impact of heat stress at older ages, to improve the antioxidative status and immune responses in growing ducks.

Keywords: *blood, immunity, thermal manipulation, Muscovy ducklings.*

INTRODUCTION

Birds can acquire thermal tolerance by embryonic or post- hatch heat manipulation. It is much difficult to conduct thermal conditioning during post hatch period compared to prenatal period in which plenty of studies on thermal conditioning during prenatal period were conducted. This concept was approved by Quinteiro-Filho *et al.* (2010) and Piestun *et al.* (2013) who indicated that heat shock activates small number of genes which were previously inactive or transcribed at low levels. Ambient temperature at certain age has a strong influence on the hypothalamic set point for temperature regulation

(Nickelmann and Tzschentke, 1997; Yahav, 2000). This balance point is defined as that temperature at which the thermoregulatory defense against heat exposure is initiated. There is evidence that the hypothalamic balance point may vary to a new higher or lower balance point according to the pre- or post-hatch thermal manipulation. Tag El-Din *et al*. (2017)

Chickens can survive under heat stress through physiological manipulation to better tolerance by acclimation to high environmental temperatures (Tzschentke and Halle, 2009) but there is a disadvantage for this acclimation, in terms of reduced body weight as a result of the reduction in feed intake. Since the implementation of thermal conditioning (Arjona *et al.*, 1988, 1990; Yahav and Hurwitz, 1996; Yahav *et al.*, 1997) can incorporate threshold changes that enable chickens to cope, within certain limits, with acute exposure to unexpected heat spells.

Several scientists have demonstrated that epigenetic adaptation can be used to induce thermotolerance in adult layer chickens, ducks and turkey (Iqbal *et al.,* 1990; Ismaiel *et al.*, 2016). However, Nickelmann and Tzschentke (2002) and Tzschentke (2002) showed that epigenetic adaptation has proximate benefits on the efficiency of thermoregulation during post- natal life of ducks. The effects were not only on chemical / metabolic thermoregulation but also on neuronal hypothalamic thermal sensitivity.

The application of higher temperature at early age increases the number of satellite cells that are necessary for muscle tissue's hypertrophy (Halevy *et al.,* 2001; Tag El-din *et al.*, 2017). The effect of higher temperature exposure at 3 day of age in broiler chicks significantly increased the number of satellite cells in compare to control group. This trend was associated considerably with IGF-I secretion (Halevy *et al.*, 2001 and El-Wardany *et al*., 2012). The fast-compensatory growth, which will occur immediately after heat stress induction, was associated with higher plasma T3 concentrations. This pattern of T3 secretion has occurred only at compensatory growth phase since after this period plasma T3 concentrations have been decreased (Yahav and Plavnik, 1999). The exposure to the thermal challenge decreased T3 and T4 levels at 21 days of rearing in broiler chicken compared to birds raised under standard thermal conditions (Pieston *et al.,* 2008).

It is well known by many investigators that acquisition of thermotolerance in birds is possible by exposing them to high ambient temperatures during critical developmental phases (i.e., the first week of life: Yahav and Hurwitz, 1996; De Basilio *et al.* 2001; Yahav and McMurty, 2001; Ismaiel *et al.*, 2016). There is also good evidence that thermoregulation in birds is controlled by changes in the temperature of thermoreceptors in the central nervous system, i.e., changes in the hypothalamus "set point" to cope with extreme environmental conditions (Yahav, 2000). In broiler chickens, heat conditioning at an early age resulted in reduction of weight gain during the first week of life, followed by an accelerated growth (Yahav and McMurtry, 2001), improved thermotolerance, and reduced mortality when re-exposed to heat stress in later life (De Basilio *et al.*, 2001; El-Monairy *et al.*, 2010). Since early thermal conditioning seems to be one of the most promising methods to improve the adaptability of broiler chickens to heat stress (Lin *et al.*, 2006).

Up to date, evaluation of the effect of early-age thermal conditioning has only been conducted in divers' studies. For instance, it is well known that HS can affect the immunity and physiological status of the animal. However, to our knowledge there were no studies reported regarding the effect of TC on growing ducks' growth, immunity and physiological status. Based on this concept, the aim of the study was to evaluate the impact of early age thermal manipulation TM, to test whether it has an overall beneficial effect, on immunity and physiological status of Muscovy ducklings.

MATERIALS AND METHODS

Birds, management and experimental design:

This research was conducted at El-Serw Waterfowl Research Station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt during the period from May to

July, 2020. A total of 200, one- day old, unsexed ducklings were randomly distributed into two equal groups (100 bird each); the first group was kept under normal brooding temperature brooded at 33 ±1°C for the first three days post hatching and served as control group (TN), while the second group was brooded under 30±1°C for the first three days then, subjected to early age thermal conditioning (TM) at 39.5 ±1°C with 55% RH for 4 h for three consecutive days (the 4^{th}, 5^{th} and 6^{th} days post hatching) by using gasoline heater. This exposure was repeated at day 80 of age, where (TN and TM) were subjected to the acute heat stress at 39.5 ±1°C and 55% (RH) for six hours only. All groups were maintained under continuous lighting program (23 h light, 1h dark) for 28 days post hatching, then subjected to daily 16 h light: 8h dark till the end of the experiment (84 days of age). Water and feed were provided *ad libitum* throughout the experimental period.

Measurements:

Growth Performance:

Live body weights (LBW) of ducks were recorded at hatch, 4, 8 and 12 weeks of age. Also, body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) were calculated.

Thermo-physiology response:

Ten ducklings from each group were selected randomly to determine the rectal temperature and respiration rate at the end of the first exposure time (day 7, T1) and at day 80 of age (T2). Rectal temperature was measured by inserting a digital thermometer in the cloacae at the depth of 3 cm. Respiration rate was measured by counting the chest movement per minute.

Blood Hematology and Biochemistry:

On days 7 and 8o of age, six ducks from each treatment were chosen around the average weight of treatment and slaughtered by severing the jugular vein. The blood sample/bird was collected in two tubes: the first one is heparinized to determine hematological parameters in terms of red blood cells (RBCs), packed cell volume (PCV %) and hemoglobin (Hb) concentration. The second tube (without anticoagulant) was centrifuged at 4000 rpm for 10 min to separate serum which was stored in sterile tubes at −20°C until the time of biochemical determinations. Immediately after collection, blood PH value was determined using digital electric pH meter. Each blood sample was divided into two portions: the first one was immediately centrifuged at 4000 rpm for 15 minutes and blood plasma was separated and stored at-20°C for further biochemical assay. Plasma lysozyme activity was measured by agarose gel lyses assay according to the method described by Schultz (1987).

The percentage of packed cell volume (PCV %) value was determined according to Hunsaker (1969), Red blood cells (RBC's) and white blood cells (WBC's) were counted in fresh blood samples as described by Natt and Herrick (1952) using hemocytometer and counted at 400 X objective of a phase contrast microscope. Differential count of 200 leucocytes was done using blood smears stained with Wright's stain and Heterophils (H) to Lymphocyte’s ratio (H: L ratio) was calculated. Serum thyroxine (T4) and Triiodothyronine (T3) concentrations were determined by RIA technique. using Gamma- Coat ^{125}I RIA Kits, Clinical Assay, Cambridge, Medical Diagnostics, Boston, MA, as reported by Britton, *et al.* (1975). Corticosterone level was determined by using RIA technique according to Sharp *et al.* (1977). Catalase (CAT) activity was determined according to Luck, (1963). Superoxide dismutase (SOD) was determined according to McCord and Fridovich (1969). Malondialdehyde (MDA) was determined according to Buege and Aust, (1978).

Statistical analysis:

Data were subjected to one – way analysis of variance using general linear model (GLM) procedure of SAS program (SAS, 1999) based on the following model:

$Yij = \mu + Ti + eij$

Where:

Yij = An observation, μ = Overall mean, Ti =Effect of treatment (1, 2), and eij = Random error.

All percentage data were subjected to arcsine transformation of the square root before statistically reanalyzed, however, the actual percentage means are presented. Significant differences among treatments means were tested by Duncan's multiple range test (Duncan, 1955) at a probability level of 0.05.

RESULTS AND DISCUSSION

Productive performance:

Live body weight (LBW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) of broiler ducks as influenced by TC during incubation are presented in Table (1).

Table (1): Effect of early age thermal manipulation and late heat exposure on productive performance of Muscovy ducks.

Age (week)	Heat treatments		
	TN	TM	Significance
	Live body weight (g)		
One day	52.8 ± 6.22	52.5 ± 4.16	NS
4 Wks.	1199.1^{b} ± 34.35	1289.5^{a} ± 41.28	**
8 Wks.	2280.8^{b} ± 45.56	2461.6^{a} ± 32.64	**
12 Wks.	3625.4^{b} ± 63.27	3944.7^{a} ± 51.59	**
	Body weight gain (g)		
1d – 4 Wks.	1145.6^{b} ± 24.17	1235.9^{a} ± 18.95	*
5 – 8 Wks.	1080.8^{b} ± 43.70	1170.8^{a} ± 37.82	**
9 – 12 Wks.	1342.5^{b} ± 36.65	1482.3^{a} ± 29.14	**
1d – 12	3570.9^{b} ± 55.37	3890.2^{a} ± 48.67	**
	Feed consumption (kg / bird)		
1d – 4 Wks.	2.225 ± 0.14	2.264 ± 0.16	NS
5 – 8 Wks.	3.368 ± 0.12	3.355 ± 0.13	NS
9 – 12 Wks.	5.046 ± 0.23	5.110 ± 0.31	NS
1d – 12 Wks.	10.639^{b} ± 0.42	10.729^{a} ± 0.45	*
	Feed conversion ratio		
1d – 4 Wks.	1.94 ± 0.01	1.84 ± 0.11	NS
5 – 8 Wks.	3.11^{a} ± 0.12	2.87^{b} ± 0.08	*
9 – 12 Wks.	$3.7 5^{a}$ ± 0.18	3.44^{b} ± 0.15	**
1d – 12 Wks.	3.15^{a} ± 0.21	2.76^{b} ± 0.14	**

a, b: Means in the same row bearing different superscripts are significantly different (P<0.05).
TN= thermoneutral temperature (control), TM= thermal manipulation (heat exposure)

Data revealed that LBW of post hatched ducklings was randomly distributed to be nearly similar for both experimental groups without significant differences. At the 4th week of age LBW of ducks was significantly ($P \leq 0.05$) higher for those exposed to TM compared by those of the control (TN) treatment. This trend was also observed for the other growing periods, where TM at early age exert significant effects on LBW of ducklings at 8 and 12 weeks of age. This was not confimed with the results obtained by Yalcin and Siegel (2003) who reported that the change in LBW of broiler chicks as a result of prehatch temperature exposure have disappeared with age. Moreover, data of Table (1) showed higher significantly ($P \leq 0.05$) increase of body weight gain for ducklings exposed to early-age thermal manipulation (TM) throughout the experimental periods: 1- 4, 5- 8 and 9- 12 weeks and for the overall period (1- 12 weeks of age) compared to the control (TN) ducklings. On the other hand, no significant effect of TM on feed consumption (FC) of ducklings throughout the entire growth period, however the cumulative FC for the whole experimental period (1- 12 weeks of age), was significantly higher for the TM ducklings compared by those of TN ones. Additionally, the ducklings exposed to heat acclimation at early age exhibited significantly better values of feed conversion ratio (FCR) during all studied periods except that from 1- 4 weeks of age, which exhibited insignificant differences between the two groups. It is well known that the FCR value is a function of feed consumption and body weight gain, hence the worse FCR values were recorded for the control group where, BWG was lower in TN compared to TM ducklings. This effect may be related to the possible influence of TM at early age on thyroid glands activity. Since, Thyroid hormones secretion and / or releasing rates acts as an adaptive response of broiler chicks , especially for those exposed to epigenetic thermal adaptation during incubation period .

Furthermore,this result is in close agreement with those reported by Sahin *et al.* (2009) who found that TM caused an increase in CRF from hypothalamic centers , and hence stimulates ACTH secretion, consequently elevated corticosterone level in blood. This hormone was known to enhance nutrients metabolism which may explain the significant increase in body weight gain of chicks at the end of the experiment. This was also confirmed by the results of Yahav (2002) who stated that thermoregulation in birds is controlled by changes in the temperature of thermoreceptors in the central nervous system, i.e., changes in the " set point " to cope with extreme environmental conditions along with the maturation of Hypothalamus – Pituitary – Adrenal (HPA) axis , which support our results.

Physiological Parameters:

Body temperature (BT) and respiration rate (RR):

Results of Table (2) showed that early age thermal manipulation (TM) had significantly increased body temperature of Muscovy ducks after the first exposure time (T2) compared to the control (TN) group (T1). However, at 80 days of age, ducks of the heat exposed group (T2) recorded significantly lower BT than that of the control group. This may be due to an adaptive response of ducklings to early heat exposure via its effect on the immature heat regulation centers located in hypothalamus, thus the thermal "set point" of hypothalamus is changed. This response was also reported previously by Osman, (1996); El-Wardany *et al.* (2012) and Tag El-Din et al. (2017) who found that body temperature is a good indicator of both heat stress and acclimation. Since, early heat exposure may cause pronounced decrease in body temperature at later age. This could be due mainly to the adaptive responses (mechanisms) prevailing homeostatic status to prevent the rise in body temperature.

Results in Table (2) clearly showed also that respiration rate (RR) of Muscovy ducks during the whole experimental period showed obvious changes. It is clear that high temperature exposure at early age caused highly significant increase in RR of TM-ducklings, which may be a short- time physiological response of ducklings to TM. However, the early heat - exposed group had low RR compared to the control one at the end of the experiment i.e., after the second exposure time (T2). This increase in RR (panting phenomenon) is desirable during severe heat spells to dissipate the excessive heat, by evaporative cooling from the respiratory passages. However, the energy cost of panting and the reduction in growth performance may be the worse consequences. Since early -age heat acclimation benefits in reducing the metabolic heat production through its effect on thyroidal hormone secretion rates or via the effect of heat acclimation on adrenal gland function, which agrees with the results of Tanizawa *et al.* (2014). It is likely that the increases of both BT and RR in the control ducks (TN) compared to TM group during high temperatures exposure at 80 days of age (T2) was stressful to ducklings and might induce hyperthermia in the ducks, which stimulate hypothalamus-pituitary- adrenal axis to counteract the negative impact of heat stress. This was confirmed by the results of Ndazo *et al.* (2012) who demonstrated that corticosterone secretion has offered protection against the adverse effects of heat stress on the BT and RR of the ducks. Moreover, some other hormones play an important role in circadian rhythm for adjustments of body temperature. For instance, melatonin reduces heat production which led to lowering body temperature and regulating heat dissipation (Zeman *et al.*, 2001). Also, melatonin may act by lowering the central set point temperature, thereby causing immediate stimulation of heat loss mechanisms.

Table (2): Effect of early age thermal manipulation and late heat exposure on body temperature and respiration rate of Muscovy ducks.

Item	Time	Heat treatment		±SE	Overall mean
		TN	TM		
Body temperature, °C	T1	41.4^{b}	41.8^{a}	0.22	41.6±0.48
	T2	42.3^{a}	41.2^{b}		41.8±0.34
Overall mean		41.9^{a}	41.5^{b}	0.19	
Respiration rate, breath /min	T1	26.5^{b}	42.4^{a}	4.65	34.4 ± 3.2^{A}
	T2	38.8^{a}	23.6^{b}		31.1 ± 3.4^{B}
Overall mean		32.7	32.9	2.67	

Aa, Bb: Means in the same row bearing different superscripts are significantly different (P<0.05).
TN= thermoneutral temperature (control), TM= thermal manipulation (heat exposure).
T1= at 7 days (after the 1st exposure time, T2 = at the end of the experiment.

Plasma thyroidal hormones:

The influence of heat acclimation on thyroid hormones (T_3 and T4) concentrations of ducks after the first (T1) and the second heat exposure time (T2) are presented in Table-3. It is clear from the results that, early heat exposure did influence significantly both hormones concentration. In this respect, T3 hormone was significantly reduced for TM ducklings after the first exposure time while thyroxine (T4) increased. Moreover, both hormones were significantly increased after late heat exposure in TM- exposed birds compared to control group. These results may infer an effect of early heat exposure on thyroid activity of the experimental ducks. Thyroid gland is involved in control of growth and development and exerts primary control of metabolic rate. Since, any treatments like heat stress that changes metabolic rate affect thyroid activity (May and McNaughton, 1980). Additionally, Tanizawa *et al.* (2014) and Tag El-Din *et al.* (2017) suggested that reduced T3 may be a part of the mechanism associated with improved thermotolerance by early age heat conditioning. Also, Arjona *et al.* (1990) implied that thermogenesis might be reduced by early thermal conditioning due to reduced thyroid activity. Reduction in circulating triiodothyronine (T3) after early thermal conditioning has been reported (Yahav and Plavnik, 1999).

Table (3): Effect of early age thermal manipulation and late heat exposure on plasma thyroid hormones (T_3 & T4) and corticosterone levels of Muscovy ducks.

Variable	Time	Heat treatments		±SE	Overall Mean
		TN	TM		
T3 (ng/dl)	T1	3.88^{a}	3.10^{b}	0.576	3.42^{B}
	T2	3.45^{b}	4.66^{a}	0.621	4.02^{A}
Overall mean		3.65	3.87		
T4 (ng/dl)	T1	15.22^{b}	17.43^{a}	1.261	16.30^{A}
	T2	13.25^{b}	16.51^{a}	1.451	14.86^{B}
Overall mean		14.20^{B}	16.95^{A}		
Corticosterone (ng/dl)	T1	3.98^{b}	5.24^{a}	0.732	4.09
	T2	5.16^{a}	3.02^{b}	0.534	3.87
Overall mean		4.57^{A}	4.13^{B}		

Aa, Bb: Means in the same row bearing different superscripts are significantly different (P<0.05).
TN= thermoneutral temperature (control), TM= thermal manipulation (heat exposure).
T1= at 7 days (after the 1st exposure time, T2 = at the end of the experiment.

Corticosterone level:

Corticosterone level of Muscovy ducks was significantly increased after the first time of heat exposure (T1), while it was decreased after heat exposure time. The elevation of corticosterone in the control group during high ambient temperature was compatible with previous researches (Geraret *et al.*, 1996). They reported marked elevation in corticosterone level following heat stress exposure. Also, Tanizawa *et al.* (2014) showed that attenuation of elevated levels of plasma corticosterone would be a cause of suppressed febrile reaction in early heat exposed chicks under heat stress. Similarly, Star *et al.* (2009) found that corticosterone levels of birds (control group) were already higher than corticosterone levels of those with “early life heat experience.” during high ambient temperature. It appears that the changes in thyroid hormones and corticosterone levels in response to early heat conditioning in this study may be part of the mechanism associated with improved thermotolerance by early age heat conditioning.

Hematological parameters:

As shown in Table (4) the overall mean of RBC’s count, hemoglobin concentration and PCV (%) of Muscovy ducks were significantly increased in early heat exposed group compared to the control group at ages studied.

There were significance differences between TN and TM groups at early and late heat acclimation in blood pH, RBC’s, PCV and Hb concentration. It is also noticed from the results that blood pH was significantly higher for TM at early heat exposure compared to control group (TN). It seems likely that the elevation of blood pH in control group was concomitant with increase in RR which is related to the carbon dioxide oxidative system that regulates the loss of bicarbonate where carbon dioxide loss is mediated by the lungs metabolism in tissues and is converted to carbonic acid through the action of the enzyme carbonic anhydrase**.** Moreover, data of Table 4 revealed that the group exposed to early (T1) and late (T2) heat exposure had significantly higher RBC' s count and hemoglobin concentration compared to the control group. Also, PCV (%) value for TN group after early heat exposure was significantly higher than the group of TMs. However, the TM group recorded higher value of PCV (%) compared to control group at late heat exposure time.

Table (4): Effect of early age thermal manipulation and late heat exposure on some hematological parameters of Muscovy ducks.

Item	Time	Heat treatments		±SE	Overall Mean
		TM	TM		
Blood pH	T1	7.12^{b}	7.26^{a}	0.12	$7.18^{B}\pm 0.06$
	T2	7.38^{a}	7.20^{b}		$7.27^{A}\pm 0.08$
Overall mean		7.24^{A}	7.16^{B}	0.09	
RBC's, $x10^{6}$ / mm^{3}	T1	3.41^{b}	3.68^{a}	0.26	$3.54^{B}\pm 0.41$
	T2	3.29^{b}	3.80^{a}		$3.85^{A}\pm 0.35$
Overall mean		3.35^{B}	3.74^{A}	0.32	
Hemoglobin concentration, g/dl	T1	10.65^{b}	12.31^{a}	1.25	$11.47^{B}\pm 1.13$
	T2	12.46^{b}	13.55^{a}		$13.01^{A}\pm 1.32$
Overall mean		11.55^{b}	12.91^{a}	1.13	
PCV, %	T1	33.24^{a}	28.23^{b}	2.27	$30.74^{B}\pm 2.54$
	T2	30.36^{b}	35.44^{a}		$32.89^{A}\pm 2.72$
Overall mean		31.79	31.83	3.08	

Aa, Bb, means in the same row bearing different superscripts are significantly different.
TN= thermoneutral temperature (control), TM= thermal manipulation (heat exposure).
T1= at 7 days (after the 1st exposure time, T2 = at the end of the experiment.

It is well documented that under heat stress conditions, birds regulate heat loss through the evaporation of water from their lungs. The most physiological consequence is that respiratory alkalosis may be developed within 60 min after the onset of acute thermal change in heat stressed hens. This respiratory alkalosis causes disruption in blood flow patterns, body water distribution, and mineral and ionic balance (Smith and Teeter, 1993) thence hematological parameters might undergo critical changes. In this concern, Brees *et al.* (1989) observed that pH increased in chickens when exposed to increasing ambient temperature. However, Siegel *et al.* (1974) found no difference in blood pH for broilers reared under continuous 35°C vs. thermoneutral conditions. Reasons for these discrepancies are not clear but may include the degree of thermal stress, type of stress, blood collection site, sampling time relative to the respiratory state of birds or early age acclimatization of birds to continuous exposure to heat stress.

The hematological data indicated decreased number of circulating RBCs and Hb concentration due to high ambient temperature. This may be due to the impact of chronic stress on iron (Fe) in broilers and the hematopoietic process (Jamadar and Jalnapurkar, 1995). This result is in accordance with the findings of Yahav *et al.* (1997), and these results are in accordance with Olayemi and Arowolo (2009) in Nigerian ducks. During summer, high ambient temperature (HAT) increases body temperature, respiration rate and respiratory water loss and oxygen consumption of birds. The increased oxygen intake increases the partial pressure of oxygen in the blood of birds (Brackenbury *et al.*, 1981) leading to decreased erythropoiesis and consequently, reducing the number of circulating erythrocyte (Donkoh, 1989). The PCV showed different pattern, in control ducks there was an increased level under high ambient temperature. On the other hand, there was a decrease in PCV in thermally- conditioned ducks. Increased PCV in controls could be attributed to increased water loss due to panting and the birds were not able to adapt. In the case of thermally conditioned birds, there was an adaptation response in the form of hemodilution to compensate water loss and an increase in the bird's ability to lose heat to the environment through water loss by evaporation without compromising plasma volume. Most of this evaporative water loss comes from the extra cellular component (Darre and Harrison, 1987).

These results agree with results obtained by Zhou *et al.*, (1998) who found that red blood cells count, and hematocrit values were greater. Also, Sahin and Kucuk (2001) found that hematocrit values were higher in heat – stressed birds compared with control group under heat stress conditions. Hypoxemia coincides with diminished oxygen saturation of the blood (Julian and Mirsalimi, 1992) along with a concurrent increase in PCV and Hb, which is a well-known physiological response to increase the oxygen-carrying capacity of blood (Luger *et al.*, 2001).

Lysozyme concentration:

Phagocytic cells are a source of lysozyme and when they are activated, lysozyme activity increases. Lysozyme plays an essential role for cell differentiation and proliferation, in providing immune-structure tissue homeostasis. Lysozyme is an enzyme with antibacterial activity that can split peptide-glycanin from bacterial cell walls particularly the gram species and it can cause lysis of the cells (Chipman and Sharon (1969).

As shown in Table (5) lysozyme activity of Muscovy ducklings were insignificantly increased in early heat exposed group compared to the control group at 12 weeks of age. The immune system guards the body against foreign substances and protects from invasion by pathogenic organisms. It can be divided into the innate system and the acquired system. Early heat exposure linearly increased the content of plasma lysozyme, which is mainly secreted during early heat exposure and can break down the polysaccharide walls of many types of by phagocytes and is a nonspecific immune effector.

The negative effect of high temperatures on the lysozyme concentration in control group could be related to a possible increase in corticosterone concentrations (Stoyanchev *et al.*, 2010) due to those glucocorticoids would induce depression of the lysozyme gene transcription, leading to the strong reduction of the circulating lysozyme concentrations. Additionally, results from Table 5 indicated that, lysozyme activity, WBC' s, heterophils (%) and H/L ratio for TM group at early heat exposed recorded higher values compared to control group. Meanwhile, no significant differences were observed between TN and TM group at T2 in respect of lysozyme activity and WBC' s. The control group recorded significantly higher values of heterophils and H/L ratio at T2 compared to heat acclimated group (TM).

Table (5): Effect of early age thermal manipulation and late heat exposure on immune responses of Muscovy ducks.

Item	Time	Heat treatments		± SE	Overall Mean
		TN	TM		
Lysozyme activity, U/dl	T1	33.15[b]	42.52[a]	4.63	37.82[B] ± 2.52
	T2	65.42	63.84		64.58[A]±4.41
Overall mean		49.27	53.15	5.32	
WBC's, X 10^3 / mm^3	T1	22.94[b]	26.56[a]	2.05	24.74[B]± 1.35
	T2	34.13	32.53		33.26[A]± 2.42
Overall mean		28.52	29.51	3.23	
Heterophils (H), %	T1	30.87[b]	39.98[a]	2.94	35.40 ± 3.13
	T2	38.59[a]	31.13[b]		34.86± 2.88
Overall mean		34.71	35.54	2.10	
Lymphocytes (L), %	T1	55.14	52.46	4.29	53.78 ± 3.94
	T2	53.48[b]	57.22[a]		55.34 ± 4.23
Overall mean		54.30	54.85	3.27	
H / L ratio	T1	0.56[b]	0.76[a]	0.12	0.65± 0.10
	T2	0.72[a]	0.54[b]		0.63± 0.09
Overall mean		0.64	0.65	0.15	

a, b: Means in the same row bearing different superscripts are significantly different.
TN= thermoneutral temperature (control), TM= thermal manipulation (heat exposure).
T1= at 7 days (after the 1st exposure time, T2 = at the end of the experiment.

Immune responses:

Data in Table (5) showed that heat treatments did not significantly affect total white blood cells count between groups. Also, the overall mean heterophils and H/L ratio of Muscovy ducks were significantly decreased in early heat exposed group compared to the control group. While, overall mean lymphocytes count of Muscovy ducks were significantly increased in early heat exposed group compared to the control group.

Heterophils are phagocytic cells designed to defend the organism against. One of the physiological responses of exposure to stress is the release of glucocorticoids, causing dissolution infections by bacteria, viruses, or foreign particles(Swenson and Reece, 1996)., while lymphocytes play an important role in immunity, particularly for the production of antibodies of lymphocytes leading to lymphopenia and there is an increase in heterophils release by the bone marrow, thus increasing their number in circulation Our findings for control birds are in accordance with those reported by Hester *et al.* (1996). The H/L ratio has been accepted as the most sensitive and reliable index for determining the effect of various stressors in poultry and other livestock (Minka and Ayo, 2011). Thus, results for H: L ratio clearly showed that early age thermal conditioning was beneficial for the birds to withstand adverse effects of high ambient temperature at marketing age. Increase of H/L ratio has been reported to reflect the effects of elevated corticosteroids in the circulation induced by stress (Kannan *et al.*, 2002). However, several mechanisms may be involved in this respect. The first possible mechanism may be the direct action (Maestroni and Conti, 1993) of early heat acclimation either on bone marrow (Kuci *et al.*, 1987) or on lymphatic tissue to accelerate leukocytogenesis (Dixon *et al.*, 1992). The second possible mechanism may be production and release of various cytokines from natural killer cells and T helper lymphocytes are enhanced an indirect action through reduction of corticosterone hormone, which was reflected by the elevation of the leukocytic count.

Antioxidant indicators:

As shown in Table (6), plasma malondialdehyde (MDA) levels of Muscovy ducks were significantly higher in TM ducklings' group after the first exposure time (T1) compared to the control group. While, SOD and CAT activities of Muscovy ducks were significantly increased after early heat manipulation compared to the control group at this time. These values were reversed when measured at the end of the experiment (T2), where MDA level was significantly decreased and the activities of both SOD and CAT enzymes increased in early- age heat conditioning group compared to the control one.

One of the key reasons for the increase of MDA levels in the control groups is the increased temperature which increases the lipid peroxidation of cells and subsequent detriments to the body. It has been reported that heat stress increases lipid peroxidation in poultry. In the current study, decreased plasma increased SOD may help to restore the balance of oxidant-antioxidant status and may enhance the ROS scavenging by elevating the concentration of SOD rather than GSH-Px.

This may be an adaptive response for ducklings against different stressor challenges at late ages. In this respect, (David *et al.* (2012) showed that a thermal exposure period relatively early in life may induce responses against oxidative stress, which support our results. Also, Abd EL-Kafy *et al.* (2008), in growing rabbits, reported that plasma antioxidative markers of early heat conditioning group were higher than that of control group during hot condition They demonstrated that rabbits exposed to heat stress showed a significantly greater increase in oxidative damage in comparison with those experiencing heat stress and, importantly, may prime the antioxidant system to withstand oxidative stress induced by heat stress in adulthood. It is also possible that expression of genes related to heat shock protein synthesis, antioxidant or DNA repair enzymes could be enhanced (Mattson and Calabrese, 2010).

Table (6): Effect of early age thermal manipulation and late heat exposure on oxidative status of Muscovy ducks.

Item	Time	Heat treatments		±SE	Overall Mean
		TN	TM		
MDA (nmol/ml)	T1	16.23[b]	25.82[a]	2.45	32.24± 2.41
	T2	48.28[a]	19.75[b]		34.04± 2.04
Overall mean		32.26[a]	22.74[b]	1.78	
CAT (U/ml/h)	T1	35.62[a]	24.13[b]	1.86	29.86[b]± 2.24
	T2	29.44[b]	52.38[a]		40.89[a]± 3.43
Overall mean		32.53 [b]	38.24 [a]	2.65	
SOD (U/ml/h)	T1	40.36[a]	36.23[b]	2.44	38.29[b]± 3.52
	T2	35.97[b]	55.69[a]		65.06[a]± 4.11
Overall mean		38.16 [a]	45.95 [b]	4.22	

a, b: Means in the same row bearing different superscripts are significantly different.
TN= thermoneutral temperature (control), TM= thermal manipulation (heat exposure).
T1= at 7 days (after the 1st exposure time, T2 = at the end of the experiment.

CONCLUSION

The current study suggests that early-age heat conditioning is beneficial to improve growth performance, blood biochemical and hematological parameters, antioxidant status and immune responses of growing Muscovy ducklings.

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تاثير التاقلم الحرارى المبكر على الأداء الإنتاجي والاستجابات الفسيولوجية والمناعية للبط المسكوفي

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هدفت هذه الدراسة الى التحقق من الآثار المفيدة للتأقلم الحراري المبكر للبط حديث الفقس على أداء النمو بعد الفقس، والتغيرات الفسيولوجية والبيوكيميائية في الدم إلى جانب الإجهاد التأكسدي والاستجابات المناعية. تم تحضين عدد 200 من كتاكيت البط بعد الفقس مباشرة (عمر يوم) علي 33 ± 1 درجة مئوية للأيام الثلاثة الأولى بعد الفقس ، وبعدها تم تقسيمها إلى مجموعتين متساويتين (100 كتكوت لكل منهما)، مجموعة للمقارنة تم تربيتها تحت درجة حرارة التحضين العادية ، بينما المجموعة الأخرى تعرضت للإجهاد الحراري المبكر (39.5 ± 1 درجة مئوية) لمدة 4 ساعات في اليوم الرابع والخامس والسادس بعد الفقس. بعد ذلك تم تحضين المجموعتان علي نفس درجات الحرارة (30 ± 1 درجة مئوية) من اليوم السابع إلى اليوم العاشر من العمر مع خفضها تدريجياً لتصل إلى 24 ± 1 درجة مئوية بحلول اليوم 28 من العمر. في بداية الأسبوع الخامس من العمر وحتى نهاية التجربة (12 أسبوعًا) تشابهت ظروف التغذية والرعاية لكل المعاملات . قبل نهاية التجربة (في اليوم 80) تم تعريض الطيور في كلا المجموعتان لدرجة حرارة مرتفعة (39.5 ± 1 درجة مئوية) ورطوبة نسبية 55% لمدة 6 ساعات. تم جمع البيانات الخاصة بالأداء الإنتاجي وكذلك قياس درجة حرارة الجسم ومعدل التنفس خلال فترة التجربة. كما تم جمع عينات الدم مرتان الأولي بعد 24 ساعة من وقت التعريض الأول، والثانية في اليوم 80 من العمر (التعريض الأخير). أظهرت النتائج أن وزن الجسم الحي و الزيادة في وزن الجسم للبط كان أعلى معنويا ($P \leq 0.05$) للبط المعرض للإجهاد الحراري مقارنة بالمجموعة الضابطة. استهلاك العلف لم يتأثر معنويا في العمر المبكر لمجموعة الإجهاد الحراري ومع ذلك وجدت زيادة في كمية العلف المأكول التراكمي بالنسبة للبط المعرض للإجهاد الحراري. تحسنت كفاءة التحويل الغذائي معنويا في للبط المعرض لـلإجهاد الحراري مقارنة بالمجموعة الضابطة. انخفضت درجة حرارة الجسم ومعدل التنفس معنويا في المرحلة الأخيرة من فترة النمو (12 أسبوعًا) نتيجة التهيئة الحرارية المبكرة. زاد نشاط الليزوزيم للبط المسكوفي في المجموعة المعرضة للاجهاد الحرارى المبكرمقارنة بالمجموعة الضابطة. كما تحسنت صورة الدم ايضا نتيجة التهيئة الحرارية المبكرة حيث لوحظت زيادة معنوية فى عددكرات الدم الحمراء ، الهيموجلوبين ، حجم المادة الخلوية مع زيادة فى عدد الخلايا الليمفاوية . وتوضح النتائج أيضا وجود زيادة معنوية في مستوي هرمونات الغدة الدرقية (T3، T4) وانخفاض معنوى فى مستوى هرمون الكورتيكوستيرون بشكل كبير في نهاية التجربة نتيجة التهيئة الحرارية. انخفض تركيز المالون داي ألدهيد في الدم بشكل ملحوظ في المجموعة المعرضة للحرارة المبكرة مقارنة بمجموعة التحكم. كما حدثت زيادة في نشاط إنزيمات Catalase ; SOD في البط المعرض للإجهاد الحراري مقارنةً بالمجموعة الضابطة. وخلصت الدراسة إلى أنه يمكن استخدام التهيئة الحرارية المبكرة لمدة 3 أيام كأسلوب عملي للتخفيف من التأثير السلبي للإجهاد الحراري علي البط في الأعمار الكبيرة ولتحسين حالة مضادات الأكسدة والاستجابات المناعية في مرحلة النمو.