Egyptian Poultry Science Journal

http://www.epsaegypt.com

ISSN: 1110-5623 (Print) – 2090-0570 (On line)

EFFECT OF EARLY HEAT EXPOSURE ON SOME PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN THE LIVER AND KIDNEY OF RABBITS BEFORE WEANING

Fadila, M. Easa and Amal, M. Hekal

Dep. of Rabb Breed Res, Animal Prod. Res. Inst., Agric. Res. Center, Min. of Agric., Dokki, Giza, Egypt

Received: 24/01/2015 Accepted: 15/02/2015

ABSTRACT: Forty five New Zealand White (NZW) rabbits were distributed into two groups as follows: The first group was kept under normal ambient temperature (25±3°C) as a control group. The second group (acclimated group) was exposed to high ambient temperature (36±3°C) at day 3post-partum for 1h for 3 consecutive days by using electric heaters. At 21 days of age each group was divided into two treatments so that, the first group includes the first and second treatments while, the second group includes the third and fourth treatments as follows: The first treatment (C) was animals kept as control without exposure to heat stress. The second treatment (HS) was subjected to high temperature (38^{o}C +0.5) and 75-80% humidity for 4 hrs (12.00-04.00 pm) daily for the period of one week till 28 days of age (the end of the experimental period). The third treatment (TA1) was kept without exposure to heat stress.

Key Words: Rabbits, Heat Stress, Antioxidant Enzymes, Histological, Liver And Kidney.

Corresponding author: drmohamedessa@yahoo.com)

The fourth treatment (TA2) was subjected to high temperature (38^{o}C +0.5) and 75-80% humidity for 4 hrs (12.00-04.00 pm) daily for the period of one week till 28 days of age (the end of the experimental period).

Cortisol, urea, creatinine, activity of aspartate (AST) and alanine (ALT) aminotransferases, the malondialdehyde (MDA) level and superoxide dismutase and catalase (CAT) activities were measured in the tissues of liver and kidney of the rabbits. The liver and kidney were collected and examined under a light microscope with hematoxylin-eosin staining. The results obtained showed that the most important results: it found that MDA levels significantly higher in liver and kidney of the heat stressed group, followed by thermal acclimatization group exposed to heat stress than thermal acclimatization group without exposed to heat stress followed by the control group.

- The antioxidant enzymes activities in the tissues of the kidneys and the liver were significantly higher for thermal acclimated group (TA1), followed by group (TA2) and the control group than heat stressed group (HS).
- Heat stress led to a marked increase in the enzymes AST, ALT, as well as in renal function measurements compared with the control group, while thermal acclimatization led to a marked improvement in the previous measurements.

Moreover, histological studies of the heat stressed group revealed dilatation of the hepatic sinusoids, interstitial hemorrhage and dilatation of the renal tubules. Notably, early thermal acclimatization completely reversed the histological changes that were induced by heat stress yielding levels that were similar to the control group. The results revealed that the early thermal acclimatization has remarkable potentials to counteract heat stress caused alterations of cortisol as well as oxidative stress biomarkers probably through their anti-stress and free radical defusing effects.It could be concluded that early thermal acclimation very effective in reducing stress-induced organ damage by inhibiting lipid peroxidation and supporting the cellular antioxidant defense system.

INTRODUCTION

High environmental temperature induces physiological stress in rabbits leading to production losses (Marai et al., 2001). Stress has been defined as a state that occurs when an animal is required to make abnormal or extreme adjustments in either its physiology or behavior in order to cope with adverse aspects of its environment and management (Fraser et al., 1975). Rabbits are homoeothermic animals and they are very sensitive to high temperatures since they have few functional sweat glands, limiting them ability to eliminate excess body heat when the environmental temperature is high.

Many organs such as the liver, kidney, and central nervous system are damaged by severe heat and thrombus infarcts, and death from heat stress may be caused by injury to these organs (Ando et al., 1997). Heat stress may lead to increased production of transition metal ions, which can make electron donations to oxygen forming superoxide or H_2O_2, which is further reduced to an extremely reactive OH radical causing oxidative stress (Zhao et al., 2006). Also, Malondialdehyde (MDA) is produced during the Reactive oxygen species (ROS) mediated peroxidation of polyunsaturated fatty acids and is a widely used marker of oxidative stress.

These active metabolites could result in drastic damage to the cell structures; protein, lipids and DNA, and further induce physiological and pathological changes, resulting in poor performance (Abdel-Kalek, 2010). In the rabbit, stress associated with exposure to high ambient temperatures decreases growth performance, possibly because of excessive production of ROS that oxidize and destroy cellular biological molecules (Liu et al., 2011). Furthermore, acute and chronic heating of cells and tissues induces alterations in nuclear and cytoskeletal structures, decrease in mitotic figures in the epithelium and somites, disruption of neural and vascular basement membranes, increase in programmed cell death; that is apoptosis, and inhibition of natural cell-mediated immunity (Katschinski et al., 2000).

Endogenous antioxidants' enzymes are the major cell defense against oxidative stress. Glutathione peroxidase (GSH-Px) and catalase (CAT) are considered the major peroxide-removing enzymes located in the cytosol. Superoxide dismutase (SOD) plays an important role in protecting against damage by the superoxide anion radical

(Chan and Decker, 1994). Glutathione peroxidase in the rabbit liver is about 6-12 folds that in cattle and sheep (Tapel et al., 1982), indicating its importance in scavenging excessive ROS produced by the tissues.

Several strategies have been recommended to ameliorate the negative effects of a high environmental temperature. The effects of heat stress can be noticeably ameliorated by heat acclimation (Yalcin et al., 2001), fortification with trace elements (Nollet et al., 2008) and dietary supplementation with vitamins (Al-Enazi, 2007). Recently demonstrated that heat acclimation (HA) improves arterial elasticity (Kaldur et al., 2013), which has been shown to be directly linked to oxidative stress and inflammation (Kals et al., 2011).

The mechanisms involved in cellular injuries caused by hyperthermia deserve more attention. Therefore, the purpose of this study was to investigate the impact of early heat exposure on histological and physiological changes and on the lipid peroxidation and some antioxidant enzymes in the heat stress-induced oxidative stress in the rabbit liver and kidney.

MATERIALS AND METHODS

Animals and Experimental protocol

This study was carried out at the Rabbits Farm of Sakha Station. Animal Production Research Institute, Agriculture Research Center, Egypt. Forty eight New Zealand White (NZW) rabbit kits randomly were divided into two groups (24 kits /group). The first group was kept under normal ambient temperature (25±3°C) as control group. The second group (Heat acclimated group) was exposed to high ambient temperature (36±3°C) at day 3 post-partum for 1h for 3 consecutive days by using electric heaters. The exposure time for heat stress was from 11:00 am to 12:00 pm to minimize the effect of circadian rhythm. Also, to avoid the stress of bereavement, the nursing mothers were returned to their newborns after each exposure for all groups. At 21 days of age each group was divided into two treatments (4 treatments x 4 kits x 3 replicates = 48 rabbits)..

The first treatment (T1) animals were kept as control.

The second treatment (T2) was subjected to high temperature (38ºC± 0.5) and 75-80% humidity for 4 hrs (12.00-04.00 pm) daily for one week till 28 days of age (the end of the experimental period).

The third treatment (Heat acclimated group) (T3) was kept without exposure to heat stress

The fourth treatment (Heat acclimated group) (T4) was subjected to high temperature (38^{o}C +0.5) and 75-80% humidity for 4 hrs (12.00-04.00 pm) daily for one week until 28 days of age (the end of the experimental period).

Blood and the tissue samples

At the end of the experimental period, blood samples of all groups were collected by venipuncture from the jugular vein into sterile vials containing anti-coagulant heparin. The plasma samples were separated by centrifugation at 3,000 rpm for 15 min and samples were frozen and stored at -20°C until plasma assay. Plasma samples of all the animals were assayed for cortisol by a radioimmunoassay (RIA) technique supplied by Immunotech, Czech Republic, urea, creatinine, activity of aspartate (AST) and alanine (ALT) aminotransferases, were estimated by the colorimetric method using commercial kits (Diamond Diagnostic, Egypt).

Also, at the end of the experimental period, feed was withdrawn from the rabbits and they were fasted for overnight with free access to water. Three rabbits from each group were slaughtered. Internal organs including kidney and liver were surgically removed. Tissue samples from each organ were divided into 2 portions. The first part of the samples wiped with filter paper and fixed in 10% neutral buffered formalin fixative for histological examination. The slides were stained with Haematoxylene and Eosin and examined morphometrically under Light Microscope. The second part was stored at –80 °C for the determination of Malondialdehyde contents and Superoxide dismutase and Catalase enzyme activities.

TISSUES HOMOGENIZATION

Tissues were homogenized in ice-cold phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Branson Sonifier 450, VWR Int. Ltd., Poole, UK) for 3 cycles (20-s sonications and 40-s pauses on ice). The homogenate was centrifuged (15,000 ×g, 10 min, 4 °C) and cell-free supernatant was subjected to enzyme assay immediately.

Catalase (CAT) assay

Catalase (CAT) activity was measured at 37 °C by following the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm ($\varepsilon 240 = 40\ M^{-1}\ cm^{-1}$) (Luck, 1963). One unit of catalase activity was defined as the amount of enzyme catalyzing the degradation of 1 μmol of

H_2O_2 per min at 37 °C and specific activity corresponding to transformation of substrate (in μmol) (H_2O_2) per minute per milligram protein.

Superoxide dismutase (SOD) assay

Superoxide dismutase (SOD) (Cu, Zn-SOD) activity in the supernatant fraction was measured using the xanthine oxidase/cytochrome method (McCord and Fridovich, 1969), where 1 U of activity is the amount of the enzyme needed to cause a half-maximal inhibition of Cytochrome C Reduction. The amount of SOD in the extract was determined as U enzyme mg–1 protein, utilizing a commercial SOD as the standard.

Malondialdehyde (MDA) assay

The analysis of lipid peroxidation was carried out as previously described (Buege and Aust, 1978) with minor modification. The reaction mixture was prepared by adding 250 μL of homogenate into 2 mL of reaction solution (15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N HCl, 1:1:1, w/v) and heated at 100 °C for 15 min. The mixture was cooled to room temperature and centrifuged (10,000 ×g for 10 min), and the absorbance of the supernatant was recorded at 532 nm. 1,1,3,3-Tetramethoxypropane was used as the MDA standard. Malondialdehyde results were expressed as nmol mg–1 protein in the homogenate.

Nucleic acid contents and total protein in tissue:Specimens of liver and kidney were collected to be examined for RNA and DNA contents. One gram of tissue is homogenized in 4 ml distilled water, out of which 1 ml is added to cold Trichloroacteic acid (TCA), centrifuged, boiled in mixture of absolute ethanol and ethanol/ether mixture 3:1. After centrifugation add 5% TCA. The supernatant is separated to be quantified using specific reagents for DNA (Diphenylamine reagent) and RNA(Orcinol reagent) according to Peares (1985) and Schneider (1957) respectively. Total protein is estimated in tissue using commercial kits according to Peter, (1968).

STATISTICAL ANALYSIS

All results were analyzed using the general linear models procedure of SAS (1999). The model was:Yij= μ+Gi+eij ; where: μ = the overall mean; Gi = effects of heat treatment and eij = residual error term. Duncan's multiple range tests was performed (Duncan, 1955) to detect significant differences among means.

RESULTS AND DISCUSSION

Cortisol levels Cortisol is the major glucocorticoid produced and secreted from adrenal cortex having profound effects on carbohydrate, fat and protein metabolism. Production and secretion of cortisol is dependent upon (ACTH) secrete from anterior pituitary, which in turn is regulated by corticotrophin releasing hormone (CRH) from hypothalamus. Cortisol plays an important role in all types of stress. The stressors induce release of cortisol by activation of the hypothalamic-pituitary-adrenal axis (Minton, 1994). The increased cortisol level in the heat stressed group in the present study suggested that the animals were exposed heat stress. Kaushish et al. (1997) also reported an increase in cortisol level during heat stress in goats. Treatment with early heat exposure decreased plasma cortisol levels, indicating that thermal acclimation may have a negative effect on cortisol levels during heat stress. The data presented in Table 1 showed that during heat stress condition, significant increase in cortisol concentration had taken place in this group compared to control group. The increase was less in heat acclimated group with heat stress followed by thermal acclimated group without heat stress. This indicates thermal acclimation reduced the stress in animals. The increase in plasma cortisol in response to high ambient temperature in hot season could be an additional factor responsible for increasing the oxidative stress in the heat stress group of rats as reflected by the decreased GSH level and SOD activity in their erythrocytes (Surekha Bhat et al., 2008). The harmful effects of superoxide anion as well as the widespread tissue damage known to occur when corticosteroids are administered in larger under heat stress than physiologic doses under normal condition. Cortisol has been shown to influence the activity of a number of dehydrogenases and oxygenases that could produce excess superoxide anion.

The decline which occurs in cortisol during the heat acclimation is attributed to the fact that it is thermogenic in animals and, consequently, the reduction of adrenocortical activity under thermal stress is a thermoregulatory protective mechanism preventing metabolic heat production in hot climate. This indicates the role of adrenal cortex gland in adaptation to stress (Alvarez and Johnson, 1993).These results indicate that thermal acclimation used in current experiment may have act as anti-stressor and

diminished the neuropathological influence of heat stress as evidenced from ameliorate cortisol level.

Liver functions:

Generally, the blood enzymes are easily and often influenced by the external environment including feeding practices, type of shelter and many other aspects of hard management, since they are intimately related to metabolism.

The levels of plasma ALT and AST activity in different groups are shown in Table 1. Heat stress produced a significant elevation in enzymes of the liver (ALT) and (AST) in this group. While, heat acclimation caused significant ($p<0.05$) decreased in these parameters compared to control animals. The acclimation followed by heat stress caused significant ($P<0.05$) decreased in ALT and AST levels and this means that heat acclimation had protective effect against the heat stress.

The results of the liver functions on rabbits subjected to heat stress showed elevation of ALT and AST activities in this group as shown in Table 1. These elevated liver enzyme activities may indicate hepatocellular damage (Mostafa, et al., 2007), which is a combined result of the high temperature and the severely reduced blood supply (Rubel, 1984). The net increase observed immediately after heat stress for AST and ALT could be also related to the net and transient increase in cortisol levels following stress (Zahran, 2004). In addition, this elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. Blood AST and ALT are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage (Naik and Panda, 2007).

These effects were coupled with a marked hepatic oxidative stress indicating liver injury. Under heat acclimation these parameters were decreased (but were still above the control level) in these groups. These results are in agreement with those of previous studies in rat (Janet et al., 1975).

Kidney functions:

Data are presented in Table 1 showed that a significant increase of urea and creatinine levels in heat stressed rabbits as compared to control group, also showed the effects of heat acclimation with or without heat stress on these parameters. They are statistically significant ($P<0.05$) lower than heat stressed rabbits and close to the control value and significantly in heat acclimated group with heat stress.

Also the results revealed that the heat acclimation was more effective in improvement the negative effects of heat stress conditions.As a measure of renal function status, serum uric acid, urea and creatinine are often regarded as reliable markers (Henry, et al.,1982 and Bonsnes and Taussky, 1982). Urea is the detoxification product of the ammonia derived from deamination of amino acids, thus urea considered to be the end product of protein catabolism (Sylvia and Mader, 1998). Creatinine is a catabolic end product, an anhydride of creatine (or phosphocreatinine) produced by loss of water (or phosphoric acid) from the molecule in an irreversible reaction (Matthews, et al., 1997). Thus, elevations in the serum concentrations of these markers are indicative of renal injury simply because the kidneys excrete them. Elevation in uric acid and creatinine in agreement with results of previous studies in buffaloes (Gudev and colleagues, 2010) who suggested that increasing plasma urea level in heat stressed buffaloes closely related with the dynamic of cortisol and blood volume fluctuation in animals under heat.

Total DNA, RNA, and total protein contents:

The products of lipids peroxidation initiate further free radical chain reactions and can cause oxidative damage to proteins and DNA (Stocker and Keaney 2004). Accumulation of oxidatively damaged DNA could lead to a decreased mRNA expression and protein production and decline in physiological functions during heat stress (Shin et al., 2008).The effects of heat stress and heat acclimation on total DNA, RNA, and total protein contents in rabbit liver and kidney are summarized in Table 2. The results showed that the values recorded for total DNA, RNA, and protein contents in both cells were significantly reduced in heat stressed group compared with the control groups. A highly significant increase was observed in heat acclimated group followed by heat acclimated group with heat stress compared to the heat stressed group as shown in Table 2. The rate of recovery was slower and did not reach the control value in heat acclimated group under heat stress. Similarly, Zhang et al. (2003) reported that the exaggerated ROS accumulation in old rats was associated with marked oxidative damage to DNA during the 24 h following hyperthermia challenge, whereas in the young rat the DNA damage peaked at 2 h. In addition, Takahashi et al. (2004) suggested that

heat-induced cell death might be dependent or associated with double-strand break formation in mammalian cells through protein denaturation. Heat-induced protein denaturation results in the disruption of centrosome-dependent mitosis (Nakahata et al., 2002) and multiple nuclear matrix-dependent functions e.g. DNA replication, DNA transcription, mRNA processing, and DNA repair (Roti Roti et al., 1998). Another possible target involved in cell death is cellular DNA because it has been reported that heat induces structural alterations and strand breaks in chromatin DNA (Takahashi et al. 2004). In the present study, heat stress induced a marked decrease in total nucleic acid content in liver and kidney cells of groups compared with control values. As DNA acts as a matrix for RNA synthesis, any modifications in DNA will affect RNA synthesis. As RNA is necessary for protein synthesis, its decrease will in turn reduce protein synthesis. This is relevant in the present study, in which a decline in total protein content was recorded in heat-stressed rabbit livers of groups as a result of a decrease in DNA and RNA contents. Similarly, Mostafa et al. (2009) reported that hyperthermia has been shown to induce a number of effects in mammalian cells including inhibition of DNA, RNA, and protein synthesis. On the other hand, oxidative DNA damage was significantly decreased in heat-acclimated subjects (Yung-Kai et al., 2012).

Hepatic and kidney tissues MDA levels:

Malondialdehyde (MDA) level was high in live and kidney in the heat stress group than all groups. The hepatic and kidney MDA levels of the control group and thermal acclimated group without heat stress were significantly lower than those of heat acclimated group with heat stress as shown in Table 3. Although the liver and kidney have the highest mass-specific oxygen consumption rates in the body, therefore coping with high rates of oxidant formation and stress (Rolfe et al., 1994), Şahin and Gumuşlu (2007) found that the most increase MDA level were in the liver and some other organs. Kim et al. (2010) have also reported a marked increase in MDA content after heat stress, which indicates that heat stress causes lipid peroxidation (LPO) by altering the expression of oxidative stress related and LPO-related transcripts. These findings are also consistent with reports indicating that lipids are the primary targets of ROS reaction in the liver (Parola et al., 1999). MDA levels were generally decreased in the thermal acclimated groups as shown in

Table 3. Relatively low levels of MDA in the thermal acclimated group may be related to the recovery capabilities of the tissues with the course of time. This may be related to the tissue-specific availability of cellular antioxidant enzymes which reduces degree of oxidant-induced degeneration during tissue damage. The reduction of the tissue MDA levels might represent a time-dependent recovery of tissue damage. These results could be suggested that the observed beneficial adaptive effect of thermal acclimation on the oxidative stress level caused by heat stress can at least partly be due to heat shock proteins. Heat shock proteins may be important modifying factors in cellular responses to a variety of physiologically relevant conditions such as hyperthermia, oxidative stress, and metabolic challenge and modifying factors in acquired thermotolerance (Kregel, 2002). It is well known that heat conditioning greatly accelerate the synthesis of the inducible HSP (especially Hsp70) (Kregel, 2002), which is thought to have both a cellular and systemic protective role (Kregel, 2002).

Hepatic and kidney tissues CAT activities:

Organisms may have an endogenous protective antioxidant defense system against the damages of free oxygen radicals. SOD, CAT and GSH-Px are enzymatic antioxidants that catalyze detoxification reactions of toxic oxygen metabolites. Catalase and GSH-Px can provide a direct defend by cleaning the hydrogen peroxide that is one of the leading hydroxyl radicals that own a potentially reactive structure (Reilly and Bulkley 1990). Results provided in Table 3 indicated that the lowest CAT activity of hepatic and kidney was detected in the heat stress group compared to the other groups. Tissue CAT activities of the thermal acclimated group were higher than those of thermal acclimated group with heat stress followed by the control group as shown in Table 3. These results were in agrees with previous works reported by Abdel-Kafy et al., (2008). Catalase is used by cells to defense against the toxic effects of hydrogen peroxide, which is generated by various reactions and environmental agents (Michiels et al., 1994). Catalase enzyme as an antioxidative enzyme was induced for protecting the whole-body against ischemia and reperfusion injury caused by hyperthermia (Yamashita et al., 1998).

Hepatic and kidney tissues SOD activities:

Superoxide dismutase constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 and O_2 which are deleterious to polyunsaturated fatty acids and proteins (Fridovich, 1975). Results illustrated in Tables 3 indicate that SOD activity of the heat stress group were lower than that of the other groups in liver and kidney tissues. Superoxide dismutase activities of the heat acclimated group without heat stress were higher than that of the control group in liver tissue., but SOD activities of the heat acclimated group with heat stress were similar with the control group in liver tissue as shown in Table 3. The results showed no significant difference in the SOD activity of kidney tissue between the control group and the heat acclimated group with or without heat stress. Additionally, SOD activity of thermal acclimated group was slightly increase than the control group followed by thermal acclimated group with heat stress in kidney tissue as shown in Table 3. These results were agrees with previous works reported by Luo et al. (2006) who reported that SOD is most sensitive to heat, and heat exposure leads to a decrease in SOD activity in rat testicular tissues. In a recent study, El-Orabi et al. (2011) reported that heat stress at 43^0C for 30 min causes a decrease in SOD-1 mRNA levels, cytoplasmic SOD protein, and enzyme activity. The immediate decrease in SOD activity following heat stress have been attributed to thermal/oxidative inactivation of the enzyme (Lushchak and Bagnyukova, 2006). The increase in plasma cortisol in response to high ambient temperature in hot season could be an additional factor responsible for increasing the oxidative stress in the heat stress group of rats as reflected by the decreased GSH level and SOD activity in their erythrocytes (Surekha et al., 2008).

Maintenance of membrane integrity is a major mechanism of heat tolerance in a number of animal species, and it is known that thermotolerance might be acquired in some species through heat acclimation; this can occur through exposure to a non-lethal heat treatment. Heat acclimation might be helpful in alleviating the membrane lipid peroxidation of animals under heat stress (Kampinga, et al, 1992). The cytoprotective functions of HSPs have been found in many organs: heart (Gray et al.,1999), brain (Yenari et al., 1999), kidney (Beck et al., 2000), intestine (Rokutan, 2000), embryo (Luft et al., 1999), etc. and that heat resistance of proteins in cellular membrane fractions is

only found under those conditions where elevated HSP72 levels are found in these membranes (Kampinga, 1993).

Histological observations

Haematoxylin and Eosin stain

Stress can disrupt the balance in an oxidant and antioxidant system and can cause oxidative damage to several tissues by altering antioxidant status, protein oxidation, and lipid peroxidation (Şahin and Gumuşlu, 2007). Hyperthermia is associated with accumulation of ROS such as superoxide anion ($O2^{-}$) and H_2O_2 (Shin et al., 2008).

Therefore, the discovery and development of potent antioxidant agents has been one of the most interesting and promising approaches in the search for treatment and prevention of oxidative stress-induced organ damage. Herein, we investigated the potency of heat acclimation on heat stress-induced microscopic alterations in the liver and kidney

Liver: The hepatic parenchyma of the control rabbits consisted of several hepatic lobules separated from each other by very delicate connective tissue septa housing the portal triad. Each hepatic lobule contained a thin walled central vein surrounded by hepatic cords radiating towards the periphery. The portal area is including a hepatic portal vein, a branch of hepatic artery and a bile ductile (Figures1and3). Treatment with heat stress caused severe liver damage including fatty changes, focal necrosis, pyknotic nuclei, karyolysis, proliferation of kupffer cells and bile ductless (Figures 2). These histopathological changes were improved in the liver of rabbits treated with heat acclimation under heat stress; few lipid droplets and little focal necrotic area (Figure 4).

Kidney: The kidney of the rabbits in control group showed the normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle consisted of tuft of blood capillaries surrounded by the Bowman's capsule. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with brush border and distal convoluted tubules lined by cuboidal cells (Figure 1 and 3).

The rabbits treated with heat stress exhibited histopathological changes; degeneration of glomerular tuft, cytoplasmic degeneration in cells of renal tubules, pyknotic nuclei, some tubules are necrotic, multiple foci of haemorrhage, dilatation and congestion of blood vessels (Figure 2). While the rabbits treated with heat acclimation under heat stress exhibited partially improvement of

glomeruli and the renal tubules. Some pyknotic nuclei were observed in the cells of renal tubules (Figure 4). Oxidative stress, which is a heat stress-induced response, has long been believed to play a role in liver and kidney damage. Although, oxidative stress has been suggested to be an important factor in tissue damage, the importance of oxidative stress has recently become more widely appreciated. Here, we investigated the effects of early heat exposure on the structural changes that were caused by heat stress.Histological examination of the liver tissues of animals that were exposed to heat stress revealed dilatation of the hepatic sinusoids and an interstitial hemorrhage. These results are in accordance with the histological lesions that were observed in the liver of broilers that were subjected to heat stress (Aengwanich and Simaraks, 2004); the authors of that study reported that the excess lipids in the hepatocytes indicated the occurrence of a sublethal injury. Most of the liver histological changes were reversed by thermal acclimation. The histological changes of liver treated with heat stress might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by heat stress. The accumulated hydroperoxidase can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxidase consequently, the basis of hepatocellular damage (Ando, et al., 1997).Histological examination of the kidney tissues of rabbits that were exposed to heat stress revealed interstitial hemorrhage, scattered renal tubules and atrophy in the glomeruli. These changes are similar to the effects of heat stress on the renal tubules of the broilers in a study by Aengwanich and Simaraks (2004), who reported that heat stress caused degeneration and necrosis of the renal tubules, followed by renal failure. The thermal acclimation during heat stress facilitated the recovery of the structure of the kidney. Consistent with our results, Elkon et al. (1980) reported damage in the proximal tubules of the subcapsular region and necrosis of tubules or glomeruli in a circumscribed area in the mouse kidney as a response to exposure to a range of hyperthermic temperatures (41°- 45°C). On its way through the kidney, this complex causes injury, mainly in the cortical region, reaching the proximal tubule and causing a gradual loss of the organ's function, these changes may be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by heat stress in the

renal tissues.In general, liver and kidney temperature approximate whole body hyperthermia due to their extensive vascular network. The liver is a sensitive organ for thermal stress (Flanagan et al., 1995). From the results of the present study on the effects of whole body hyperthermia on tested normal tissues and biochemical parameters, liver appeared to be at high risk of significant injury.

Conclusion

In conclusion, results show that the stress inducing factors affect various organs to different degrees. The cellular and biochemical reactions of some physically close organs, like the liver and kidney, might be different from each other. Additionally, results show that exogenously administered early thermal acclimation improves stress induced cellular damage and antioxidant enzyme systems on different levels for different organs. Thus, the early thermal acclimation might be most useful during heat stress conditions in order to protect organs from stress-induced cellular damage.

Table (1): Effect of early thermal acclimation on some physiological parameters in rabbits during heat stress.

Items	C	HS	TA1	TA2	SE
Cortisol (ng/ml)	6.04^{c}	10.84^{a}	7.47^{b}	8.40^{b}	±0.45
AST (U/l)	18.23^{c}	27.66^{a}	15.13^{d}	21.82^{b}	±0.26
ALT(U/l)	36.52^{c}	48.62^{a}	30.39^{d}	41.34^{b}	±0.27
Urea (mg/dl)	22.19^{c}	31.88^{a}	17.47^{d}	25.43^{b}	±0.77
Creatinine (mg/dl)	1.81^{b}	2.58^{a}	1.22^{c}	2.04^{b}	±0.17

$^{a, b....}$Means with different superscripts within raw are significantly different ($P \leq 0.05$).

C= Control group – HS= heat stress group – TA1= thermal acclimation without exposure to heat stress – TA2= thermal acclimation with exposure to heat stress AST=aspartate aminotransferase

ALT=alanine aminotransferase

Table(2): Effect of early thermal acclimation on total DNA, RNA, and total protein contents in different tissues of rabbits during heat stress.

Items	C	HS	TA1	TA2	SE
DNA in liver (mg/g)	0.41^{a}	0.30^{c}	0.40^{a}	0.36^{b}	±0.01
RNA in liver (mg/g)	0.25^{a}	0.20^{b}	0.25^{a}	0.24^{a}	±0.01
Total protein contents in liver (mg/g)	7.04^{a}	5.33c	7.08^{a}	6.38^{b}	±0.13
DNA in kidney (mg/g)	0.36^{b}	0.26^{d}	0.38^{a}	0.33^{c}	±0.01
RNA in kidney (mg/g)	0.25^{a}	0.18^{b}	0.23^{a}	0.23^{a}	±0.01
Total protein contents in kidney (mg/g)	6.65^{a}	4.97^{b}	6.60^{a}	6.02^{a}	±0.34

$^{a, b....}$Means with different superscripts within raw are significantly different ($P \leq 0.05$).

C= Control group – HS= heat stress group – TA1= thermal acclimation without exposure to heat stress – TA2= thermal acclimation with exposure to heat stress. DNA= deoxyribonucleic acid. RNA=ribonucleic acid

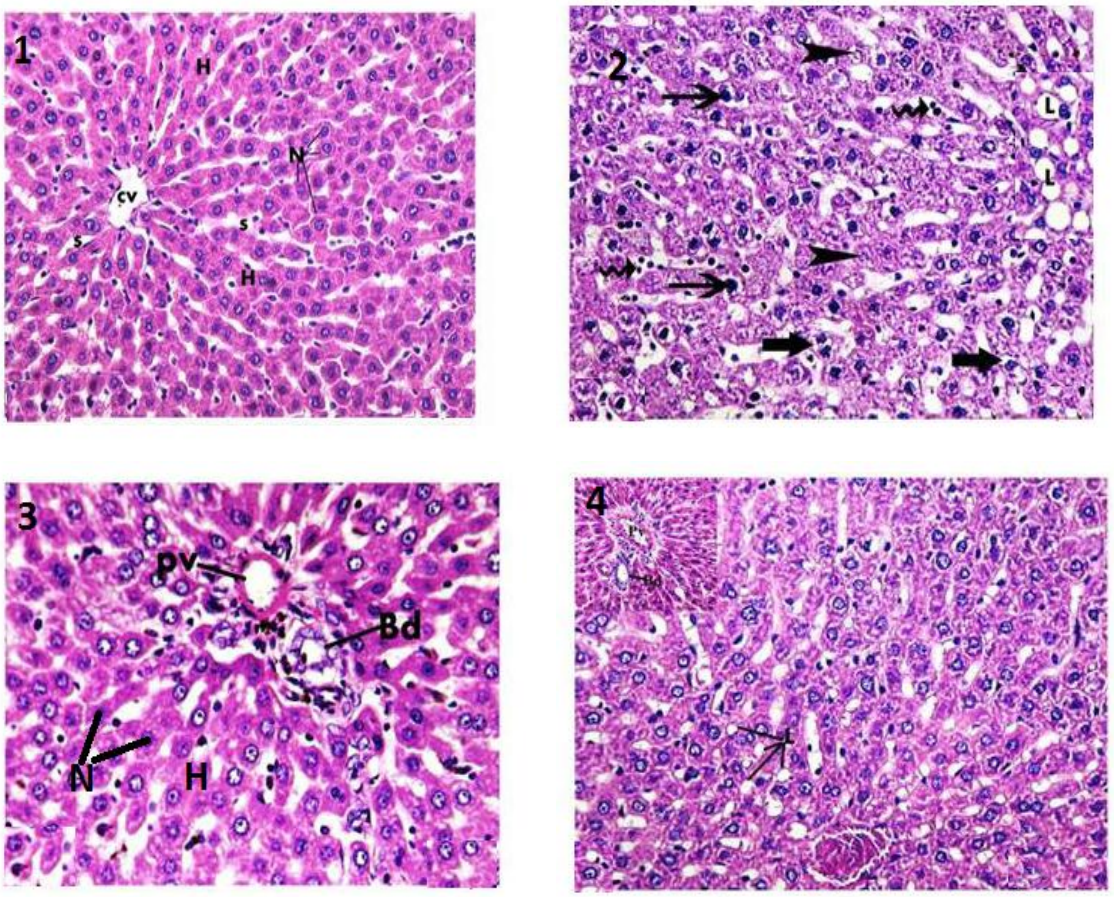
Table(3): Effect of early thermal acclimation on oxidative statues in different tissues of rabbits during heat stress.

Items	C	HS	TA1	TA2	SE
MDA in liver (nomol/mg protein)	50.36^{c}	74.59^{a}	50.03^{c}	60.77^{b}	±4.74
MDA in kidney (nomol/mg protein)	45.27^{c}	59.58^{a}	44.74^{c}	55.81^{b}	±4.37
SOD in liver (U/mg tissue)	3.04^{b}	1.96^{c}	3.75^{a}	3.12^{b}	±0.15
SOD in kidney(U/mg tissue)	2.83^{a}	1.87^{b}	2.86^{a}	2.61^{a}	±0.16
CAT in liver(U/mg tissue)	7.20^{c}	5.39^{d}	9.22^{a}	8.30^{b}	±0.22
CAT in kidney(U/mg tissue)	6.12^{b}	4.31^{c}	8.38^{a}	7.32^{a}	±0.67

$^{a, b....}$Means with different superscripts within raw are significantly different ($P \leq 0.05$).

C= Control group – HS= heat stress group – TA1= thermal acclimation without exposure to heat stress – TA2= thermal acclimation with exposure to heat stress. MDA= Malondialdehyde.

SOD = Superoxide dismutase CAT= Catalase



Figures 1, 2, 3 and 4: Sections in the liver stained with H and E showing: (1 and 3); hepatic tissue of control and early acclimated rabbit groups showing normal hepatic architecture, hepatocyte (H), with their normal nuclei (N), sinusoids (s) and central vein (cv). Also, portal tract with bile ductless (Bd) and portal vein (pv) is observed, respectively. Hepatic tissues of heat stress treated group showing (2); loss of hepatic architecture and many fatty droplets (L), vacuolated cytoplasm (thick arrows), pyknotic nuclei (thin arrows), karyolysis (head arrows), proliferation of kupffer cells (irregular arrows), and (4); Hepatic tissue of rabbit treated with (heat acclimation under heat stress) showing partially improvement of hepatocyte, few lipid droplets (L), hyalinization area around portal vein (pv) and proliferation of bile ductless (Bd). Original magnification of each figure; x 200.

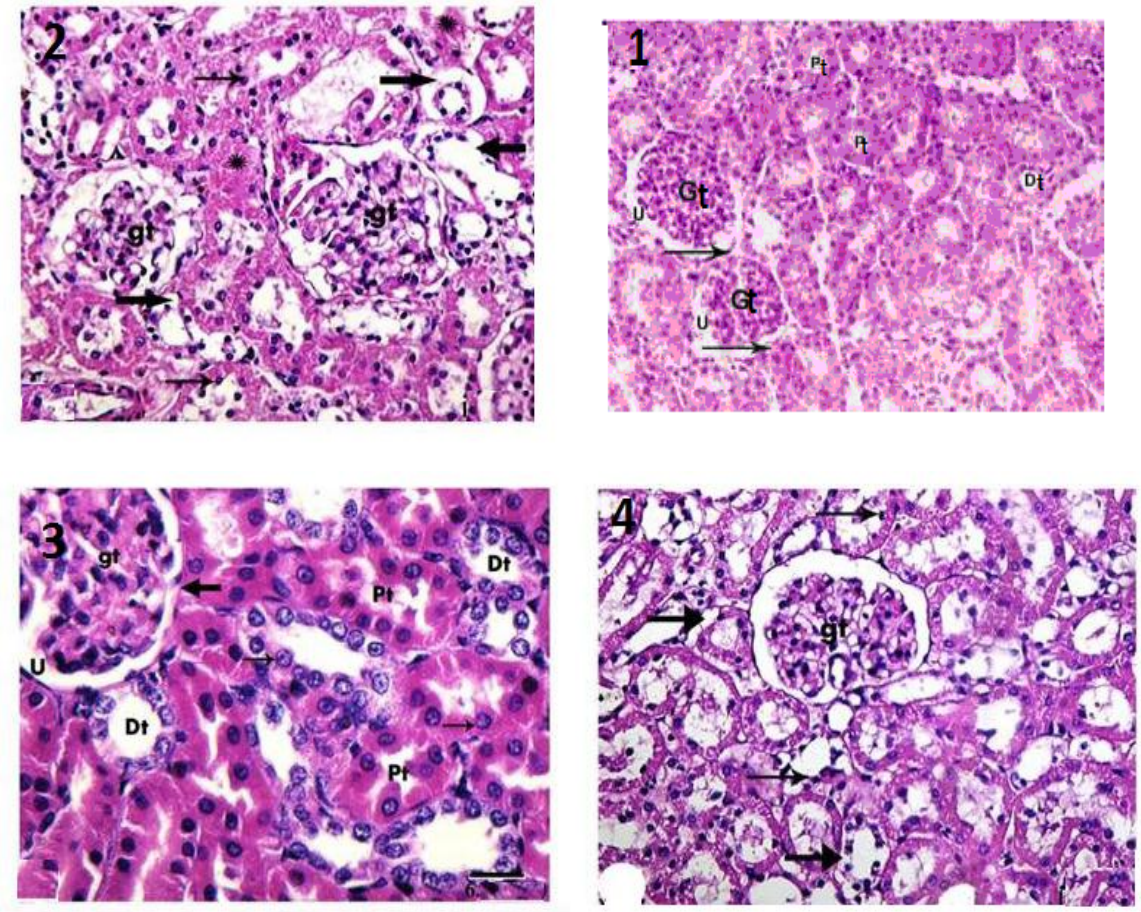


Figure 1, 2, 3 and 4. Photomicrographs of sections in the cortex of kidney stained with H and E showing (1 and 3); renal tissue of control and heat acclimated groups demonstrating normal appearance of glomerular tuft (gt), urinary space (U), Bowman's capsule (thick arrow), proximal tubule (pt), distal tubules (Dt) with their nuclei (thin arrows). Renal tissue of heat stress treated rabbit showing (2); disrupted of Bowman's capsule, degenerated cytoplasm of some cells of renal tubules (thick arrows), some tubules are necrotic (*), pyknotic nuclei (thin arrows) and (4); renal tissue of rabbit treated with heat acclimation under heat stress showing improvement of glomerular tuft, degenerated cytoplasm of some cells of renal tubules (thick arrows) and some pyknotic nuclei (thin arrows). Original magnification of each figure; x 200.

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الملخص العربى

تاثير التعريض الحرارى المبكر للارانب قبل الفطام على بعض التغييرات الهستولوجية والفسيولوجية في الكبد والكلية

فضيلة محمد عيسى ، أمل مغاورى هيكل

قسم بحوث تربية الأرانب والرومى والطيور المائية، معهد بحوث الإنتاج الحيوانى، مركز البحوث الزراعية، الدقى، جيزه، مصر

لقد أظهرت دراسات سابقة أن الإجهاد الحراري يمكن أن يؤدي إلى تلف الأنسجة واختلال فى وظائف الاعضاء. لذ تهدف هذه الدراسة للتعرف على الآثار السلبية للإجهاد الحراري والدور الوقائي للاقلمة الحرارية من التغيرات الفسيولوجية والنسيجية بسبب الإجهاد الحراري. تم توزيع خمسة وأربعين أرانب إلى مجموعتين على النحو التالي: المجموعة الأولى كانت تحت درجة الحرارة الطبيعية (٢٥ درجة مئوية ± ٣). بينما تعرضت المجموعة الثانية لارتفاع درجة الحرارة (٣٦ درجة مئوية ± ٣) وذلك في اليوم الثالث بعد الولادة لمدة ٣ أيام متتالية باستخدام الدفايات الكهربائية. وعند ٢١ يوم من العمر تم تقسيم كل مجموعة إلى معاملتين بحيث تكون المجموعة الأولى تشمل المعاملة الاولى والثانية اما المجموعة الثانية تشمل المعاملة الثالثة والرابعة على النحو التالي:

- المعاملة الأولى (C) ظلت ككنترول دون التعرض للإجهاد الحراري.
- المعاملة الثانية (HS) لدرجة حرارة عالية (٣٨ درجة مئوية ± ٠,٥)اما نسبة الرطوبة ٧٥-٨٠٪ لمدة ٤ ساعات (١٢,٠٠-٠٤,٠٠ ظهرا) يوميا لمدة أسبوع واحد حتى ٢٨ يوما من العمر
- المعاملة الثالثة (TA1) ظلت ككنترول سلبى دون التعرض للإجهاد الحراري.
- المعاملة الرابعة (TA2) لدرجة حرارة عالية (٣٨ درجة مئوية ± ٠,٥)اما نسبة الرطوبة ٧٥ -٨٠٪ لمدة ٤ ساعات (١٢,٠٠-٠٤,٠٠ ظهرا) يوميا لمدة أسبوع واحد حتى ٢٨ يوما من العمر.
- تم تقديرالمقاييس التالية هرمون الكورتيزول، ومحتوى المالوندالديهايد (MDA) malondialdehyde وانزيمات الكاتالاز (CAT) – (SOD) فوق أكسيد الديسميوتاز في أنسجة الكبد والكلية للأرانب .
- من اهم النتائج المتحصل عليها: وجد أن مستويات MDA اعلى معنويا فى الكبد والكلية لمجموعة الإجهاد الحراري(HS) يليها مجموعة التأقلم الحراري المتعرضة للإجهاد الحراري مقارنة بمجموعة التأقلم الحراري بدون تعريض للإجهاد الحراري يليها مجموعة الكونترول.
- كانت انشطة الانزيمات المضادة للاكسدة في انسجة الكلى والكبد أعلى معنويا للمجموعة التأقلم الحرارى (TA1)يليها مجموعة (TA2) والكنترول مقارنة بمجموعة للإجهاد الحراري (HS).
- الإجهاد الحراري أدي إلى ارتفاع ملحوظ في انزيمات AST و ALT وكذلك فى مقاييس وظائف الكلية مقارنة مع مجموعة الكونترول ، في حين التأقلم الحرارى أدي إلى تحسن ملحوظ في المقاييس السابقة.
- كشفت الدراسات النسيجية للمجموعة الإجهاد الحراري توسع الجيوب الكبدية، ونزيف دموى داخل الخلية وتوسع من الأنابيب الكلوية. والجدير بالذكر أن التاقلم الحرارى في وقت مبكر عكس تماما التغيرات النسيجية التي يسببها الإجهاد الحراري التي كانت مشابهة لمجموعة الكونترول.
- وكشفت النتائج أن التأقلم الحراري في وقت مبكر لديه إمكانيات رائعة لمواجهة الإجهاد الحراري بسبب تغييرات مستوى الكورتيزول وكذلك المؤشرات الحيوية للاجهاد التاكسدى لها وذلك ربما انة يعمل كمضاد للاجهاد وكذلك فى الحماية من الشقوق الحرة المتكونة .

لذا توصى الدراسة بان التأقلم الحراري في وقت مبكر فعالة جدا في الحد من الأضرار التي يسببها الإجهاد الحرارى عن طريق تثبيط بيروكسيدات الدهون ودعم منظومة الدفاع الخلوي المضادة للأكسدة.