

# CHANGES IN OXIDATIVE PROFILE, ACTIVITY OF SOME GASTROINTESTINAL ENZYMES AND PERFORMANCE OF GROWING RABBITS DURING HOT SEASON DUE TO NEONATAL HEAT EXPOSURE

Abdel-Kafy E.M.\*, Hoda A.S., Saeed A.M.

Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt

\*Corresponding author:sayedabdcaffy@yahoo.com

## ABSTRACT

The aim of this study was to examine the effect of neonatal heat exposure treatment on oxidative stress profile, some gastrointestinal enzymatic activities and performance of growing rabbits during hot (summer) season. Fifty New Zealand White (NZW) kits were divided into two groups (25 kits/group). In the control group (C), kits were kept under normal ambient temperature ( $21\pm 2^{\circ}\text{C}$ ). In the neonatal heat exposure group (H), kits were exposed to high ambient temperature ( $34\pm 2^{\circ}\text{C}$  for 6 hours) at day 5 post-partum. All kits were weaned at 30 days of age. The total antioxidant capacity in plasma of neonatal heat exposure group was significantly ( $P<0.05$ ) higher than that of control group under hot condition. Nitric oxide (NO) significantly decreased in neonatal heat exposure group. Specific activities of amylase, protease and lipase in the small intestine of H kits were higher compared with those of C group. Neonatal heat exposure improved daily feed intake and feed conversion compared with control group. There was no difference in weight of kits at weaning between groups. The body weights of young H rabbits at post-weaning and at 10 weeks of age were higher than those of C group. In conclusion, neonatal heat exposure improved weight of young rabbits when reared under hot condition. This may be due to increasing total antioxidant capacity with decreasing the nitric oxide at cellular level. Moreover, neonatal heat exposure may improve the efficiency of enzymes activity in the gastrointestinal tract of rabbit.

**Key words:** Neonatal heat exposure, Oxidative stress profile, Gastrointestinal enzymes.

## INTRODUCTION

Several studies indicated that after exposure to heat, plasma levels of super-oxide anions, hydrogen peroxide and nitric oxide increased, as well as increased lipid per-oxidation products that can be found in various cell lines and tumor tissue (Matsumoto *et al.*, 1999). Free radicals and intracellular metabolic oxidation/reduction (redox) reactions have been implicated in such phenomenon. Superoxide dismutase, catalase and glutathione peroxidase are the main cellular reactive oxygen species (ROS) degrading enzymes. Superoxide dismutase converts the superoxide radical into hydrogen peroxide, which is metabolized by catalase and glutathione peroxidase (Lenehan *et al.*, 1995). In hyperthermal condition, heat shock proteins (HSPs) may be induced as a cellular defense, which would support the concept of an inverse relationship of HSP induction and heat-induced cytotoxicity (Riabowol *et al.*, 1988). Liew *et al.* (2003) reported that the improved heat tolerance and disease resistance by early age feed restricted or /and heat exposed birds could be attributed to the best HSP 70 response. Also, some studies indicate that maternal short-term heat exposure and neonatal short-term heat exposure led to lower weight at birth and weaning, but it did not result in lower pre- and post- weaning mortality rate, heavier weight and higher growth rate in growing rabbits during hot weather than those in control group (Abdel-Kafy, 2006). Similar trends were found in ducks (El-Badri *et al.*, 2007) and in chickens (Yahav and Plavnik, 1999).

The effect of neonatal heat exposure on the oxidative stress profile and gastrointestinal tract enzymes has not been studied. The purpose of the present study was to examine the effect of neonatal heat exposure treatment on oxidative stress profile, some gastrointestinal enzymes activity and performance of growing rabbit during hot season.

## MATERIALS AND METHODS

### Animals and experimental design

This work was carried out in a commercial rabbitry located in Cairo Governorate, Egypt from May to August, 2006 (hot season). Fifty New Zealand White (NZW) rabbit kits were divided into two groups (25 kits/group). The first group (Control group, C) was kept under normal ambient temperature ( $21\pm 2^{\circ}\text{C}$ ) as control group. The second group (Heat group, H) was exposed to high ambient temperature ( $34\pm 2^{\circ}\text{C}$  for 6 h) by using electric heaters at day 5 post-partum. All kits in both groups were weaned at 30 days of age.

After weaning, kits were housed in individual cages. Cages were equipped with feeding hoppers made of galvanized steel and have nipples for automatic drinking. The internal minimum and maximum ambient temperatures and relative humidity throughout the experimental period were  $20.6^{\circ}\text{C}$  and  $34.3^{\circ}\text{C}$ , and the relative humidity were 47.2% and 75.7%, respectively. Kits were fed ad-libitum on a commercial pelleted diet. The chemical composition of diet was: 16.4% crude protein, 13.3% crude fiber, and digestible energy of 10.45 MJ/kg diet. Weights of kits and feed intake were recorded at weekly intervals from weaning up to the 10<sup>th</sup> week of age.

### Plasma and tissue sampling

Four kits from each group were sacrificed at the 10<sup>th</sup> week of age. Blood samples were collected in tubes containing EDTA. Plasma was separated by centrifugation of blood samples at speed of 3000 r.p.m. for 15 minutes. Plasma was stored at  $-80^{\circ}\text{C}$  until assaying oxidative profile parameters. Gastrointestinal tract was removed to collect the contents of the small intestine and caecum. Samples were stored at  $-80^{\circ}\text{C}$  until enzymes assays. Liver was removed and tissue samples were stored at  $-80^{\circ}\text{C}$  until catalase activity determination.

### Oxidative stress parameters

Parameters of oxidative stress profile were assayed by colorimetric technique using commercial kits (Biodiagnostic, Egypt). Total antioxidant capacity (mmol/l) in plasma and activity of catalase (U/g) in liver were determined according to Koracevic *et al.* (2001) and Aebi (1984), respectively. Oxidative damage parameters in plasma included lipid-peroxide (nmol/ml), nitric oxide ( $\mu\text{mol/l}$ ) and hydrogen peroxide (mmol/ml) were determined as described by Ohkawa *et al.* (1979), Schabel *et al.* (1961) and Aebi (1984), respectively.

### Enzyme activity assays

All enzyme activities were assayed in contents both of small intestine and caecum colorimetrically as following: amylase, cellulase and pectinase activity (U/ml) were assayed using starch, CM-cellulose and sodium polypectate as substrates, respectively, according to Somogyi (1952). Protease (U/ml) was assayed by using azo-casein as a substrate according to Chopra and Mathur (1983). Lipase (U/ml) was assayed by colorimetric technique using kit of Biodiagnostic (Egypt).

### Statistical analysis

All results were analyzed using the general linear models procedure of SAS (1999). The model was:  $Y_{ij} = \mu + G_i + e_{ij}$ ; where:  $\mu$  = the overall mean;  $G_i$  = effects of heat treatment and  $e_{ij}$  = residual error term.

## RESULTS AND DISCUSSION

### Oxidative stress profile

Levels of both lipid-peroxide (nmol/ml) and hydrogen peroxide (mmol/ml) were not significantly different between control and neonatal heat exposure group (Table 1). However, nitric oxide (NO) was significantly higher in control than in neonatal heat exposure group. This result is consistent with findings of Pittet *et al.* (2002), who reported that stress preconditioning decreased the production of NO. Level of hydrogen peroxide (as a source for ROS generation) in control group was nearly equal to that of heat group, but it might be affected by HSP which is more induced in the heat group as proved by Abdel-Kafy (2006) in rabbit. This explanation may be supported by a significant decrease of NO in the neonatal group (Table 1) which may be due to the functions of HSP families under stress conditions.

**Table 1:** Oxidative damage parameters and total antioxidant capacity in plasma of NZW rabbits at 10 weeks of age

	Control	Heat	± SE
Oxidative damage parameters:			
Lipid-peroxide (nmol/ml)	13.5	15.7	2.03
Nitric oxide (µmol/l)	12.5 <sup>a</sup>	5.5 <sup>b</sup>	0.47
Hydrogen peroxide (mmol/ml)	9.35	9.60	1.67
Total antioxidant capacity (mmol/l)	2.13 <sup>b</sup>	2.24 <sup>a</sup>	0.024

<sup>a,b</sup> Values having different superscripts in the same row are significantly different (P<0.05)

Total antioxidant capacity (mmol/l) in plasma of H group was significantly higher than that in plasma of C group under hot condition. The partial resistance to hyperthermia was correlated to the higher antioxidative capacity of the apoptosis cells as reported in rats by Dörthe *et al.* (2000).

Activity of catalase (U/g) as detoxifying enzyme was not significantly different between control and heat group (Table 1). The activation of anti-ROS enzymes induced by hyperthermia was important in the acquisition of cardioprotection against ischemia/reperfusion injury in rats (Yamashita *et al.*, 1998).

### Small intestine and caecal enzymes

Activities (U/ml) of amylase, protease and lipase enzymes in small intestinal contents of H group were higher compared with C group (Table 2). The same trend was found by Uni *et al.* (2001), who reported that chicks in heat exposure group at day 3 of age increased the activity of small intestinal enzymes compared to control group. This result may be attributed to higher mRNA expressions that translated to the new enzymes synthesis and also to an elevation in the digestive and absorptive capacity (Uni *et al.*, 2001).

Neonatal heat exposure led to significant elevation in pectinase activity in caecum compared with control, however, the activity of cellulase was not significantly affected (Table 2). Information on rabbit caecal pectin-degrading enzymes is not complete, except on enzymes of *Bacteroides caccae* (Sirotek *et al.*, 2004).

**Table 2:** Activities (U/ml) of some enzymes in contents of the small intestine and caecum of NZW rabbit at 10 weeks of age

	Control	Heat	± SE
In small intestine			
Amylase	0.58 <sup>b</sup>	1.41 <sup>a</sup>	0.12
Protease	0.071	0.074	0.002
Lipase	0.077	0.083	0.009
In caecum			
Cellulase	0.52	0.42	0.054
Pectinase	0.28 <sup>b</sup>	0.41 <sup>a</sup>	0.028

<sup>a,b</sup> Values having different superscripts in the row are significantly different (P<0.05)

### Feed intake and feed conversion

Neonatal heat exposure increased daily feed intake compared with control group from weaning to the 10<sup>th</sup> week age (Table 3). Also, positive linear correlation was found in chickens and turkeys between early-age thermal exposure and feed intake (Yahav *et al.*, 1999).

**Table 3:** Daily feed intake and feed conversion from weaning (30 days) to 10<sup>th</sup> week of age

	Control	Heat
Daily feed intake (g/d)	121.34	123.39
Feed conversion	3.47	3.14

Feed conversion in neonatal heat exposure group was improved by 10.5% compared to control group (Table 3). Improving feed conversion might be due to the association between early-age thermal conditioning, feed intake and plasma T<sub>3</sub> levels which was reflected in better intestinal capacity to proliferate, grow and digest nutrients (Yahav *et al.*, 1997). Also, this result may be due to increasing enzymes activity in the small intestine and caecum (Table 2).

### Body weight

There was no difference in weight of kits at weaning (Table 4). The weaning in rabbits is often associated with lower weight gain and in some instance with diarrhoea and morbidity (Ozimba and Lukefahr, 1991), but in the present study the body weights of H kits in the 1<sup>st</sup> and the 2<sup>nd</sup> week post-weaning were higher than those of C kits. Also, in the 10<sup>th</sup> week of age, the body weights of rabbits in heat group were higher than of control group (Table 4). Similar results were found by Abdel-Kafy (2006), who reported that kit weights in neonatal heat exposure group were higher than in control by 65 and 70 g approximately at 4 week post weaning and during the growth period, respectively. The broiler chicks exposed to heat and feed restriction on day 4 or 5 of age had HSP 70 density and weight gain higher than control chicks (Liew *et al.*, 2003). These results are in disagreement with findings of May (1995), who reported that early heat exposure did not influence the body weight gain, feed efficiency, growth or mortality.

**Table 4:** Body weights (g) at weaning, post-weaning and in the 10<sup>th</sup> week of age

	Control	Heat
Body weight at weaning	574.0± 29.8	538.3±30.5
Body weight at 1 week post-weaning	678.1±28.6 <sup>b</sup>	785.0±28.6 <sup>a</sup>
Body weight at 2 week post-weaning	865.1±46.1	908.1±46.4
Body weight at 10 week of age	1986.1±41.2	2085.0±41.2

<sup>a,b</sup>Values having different superscripts in the same row are significantly different (P<0.05)

## CONCLUSIONS

In conclusion, neonatal heat exposure improved growth of rabbits under hot condition. This may be due to increasing total antioxidant capacity with decreasing nitric oxide concentration at the cellular level after neonatal heat exposure. Also, neonatal heat exposure may lead to the improvement in the efficiency of enzyme activity in the gastrointestinal tract and consequently to the improvement of the feed conversion.

## ACKNOWLEDGEMENTS

My sincere appreciation and more thanks to Dr. Saeed Mansour in Solid and Water Research Institute for his sincere advice, providing me the necessary facilities and help me to complete enzymes assay.

## REFERENCES

- Abdel-Kafy E.M. 2006. Effect of heat exposure and feed restriction as bio-stimulation method for enhancing rabbit productivity. *Ph.D. Thesis, Agri. Univ., Giza, Egypt.*
- Aebi H. 1984. Catalase in vitro. *In Methods Enzymol., 105, Academic Press, 121-126.*
- Chopra A.K., Mathur D.K. 1983. Factors affecting protease production by *Bacillus stearothermophilus*. *J. Food Protect., 116, 1020-1025.*
- Dörthe M., Katschinski S., Kristina B., Schindler, Susann G., Joachim F. 2000. Pivotal role of reactive oxygen species as intracellular mediators of hyperthermia-induced apoptosis. *J. Biol. Chem., 275, 21094-21098.*
- El-Badri A.S., El-Kholy K.H., Hassanin Mahrosa M. 2007. Effect of early-age acclimation on development of small intestine and growth performance in muscovy ducks during summer season. *In: Proc. 4<sup>th</sup> World Poultry Conference, 2007 March, Sharm El-Sheik, Egypt.*
- Koracevic D., Koracevic G., Djordjevic V., Andrejevic S., Cosic V. 2001. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol., 54, 356-361.*
- Lenehan P.F., Gutierrez P.L., Wagner J.L., Milak N., Fisher G.R., Ross, D.D. 1995. Resistance to oxidants associated with elevated catalase activity in HL-60 leukemia cells that overexpress multidrug-resistance protein does not contribute to the resistance to daunorubicin manifested by these cells. *Cancer Chemother. Pharmacol., 35, 377-86.*
- Liew P.K., Zulkifli I., Hair-Bejo M., Omar A.R., Israf D.A. 2003. Effects of early age feed restriction and heat conditioning on heat shock protein 70 expression, resistance to infectious bursal disease, and growth in male broiler chickens subjected to heat stress. *Poult. Sci., 82, 1879-1885.*
- May J.D. 1995. Ability of broilers to resist heat following neonatal exposure to high environmental temperature. *Poult. Sci., 74, 1905-1907.*
- Matsumoto H., Hayashi S., Hatashita M., Ohnishi K., Ohtsubo T., Kitai R., Shioya H., Ohnishi T., Kano E. 1999. Nitric oxide is an initiator of intercellular signal transduction for stress response after hyperthermia in mutant p53 cells of human glioblastoma. *Cancer Res., 59, 3239-3244.*
- Ohkawa H., Ohishi N., Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem., 95, 351-358.*
- Ozimba C.E., Lukefahr S.D. 1991. Comparison of rabbit breed types for postweaning litter growth, feed efficiency, and survival performance traits. *J. Anim. Sci., 69, 3494-500.*
- Pittet J.F., Lu L.N., Geiser T., Lee H., Matthay M.A., Welch W.J. 2002. Stress preconditioning attenuates oxidative injury to the alveolar epithelium of the lung following haemorrhage in rats. *J. Physiol., 538, 583-597.*
- Riabowol K.T., Mizzen L.A., Welch W.J. 1988. Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70. *Science, 21, 242, 433-436.*
- SAS 1999. SAS user's guide: Statistical Analysis System Institute, SAS Inst. Inc., Cary NC, USA.
- Schabel F.M., Montgomery J.A., Skipper H.E., Laster W.R., Thomson J.R. 1961. Experimental evaluation of potential anticancer agents. I. Quantitative therapeutic evaluation of certain purine analogs. *Cancer Res., 21, 690-699.*
- Sirotek K., Slováková L., Kopečný J., Marounek M. 2004. Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rabbit caecal bacterium *Bacteroides caccae*. *Lett. Appl. Microbiol., 38, 327-332.*
- Somogyi M. 1952. Notes on sugar determination. *J. Biol. Chem., 195, 19-23.*
- Uni Z., Gal-Garber O., Geyra A., Sklan D., Yahav S. 2001. Changes in growth and function of chick small intestine epithelium due to neonatal thermal conditioning. *Poult. Sci., 80, 438-45.*
- Yahav S., Shamai A., Habersfeld G., Horev S., Hurwitz Friedman E.M. 1997. Induction of thermotolerance in chickens by temperature conditioning: heat shock protein expression. *In: C. M. Blatteis (Ed.) An Update in Thermoregulation from Cellular Functions to Clinical Relevance. New York Academy of Science, New York, USA, 628-636.*
- Yahav S., Plavnik I. 1999. Effect of early age thermal conditioning and food restriction on performance and thermotolerance of male broiler chickens. *Br. Poult. Sci., 40, 120-126.*
- Yamashita N., Hoshida S., Taniguchi N., Kuzuya T., Hori M. 1998. Whole-body hyperthermia provides biphasic cardioprotection against ischemia/reperfusion injury in the rat. *Circulation, 6, 1414-21.*

