

Beni-Suef University Faculty of Science Zoology Department



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# Molecular and immunological studies on tolerance to heat stress in growing rabbits

A Thesis submitted for: **Fulfillment of Ph.D. in Science** in Zoology (Immunology) **by** 

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2020

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#### Abstract

The drastic impacts of heat stress (HS) on rabbit's farms cannot be ignored. The goal of this work is to investigate the effects of chronic HS on immune system and on organs' function, and to evaluate the alleviating potential of diet supplements of Moringa oleifera leaf extract (MOE), vitamin (Vit) C, and sodium bicarbonate (NaHCO<sub>3</sub>) on HS-induced alterations in NZW rabbits. To achieve this goal, a total of 45 growing male NZW rabbits were divided into five groups and were designed as control (group 1), HS (group 2), HS+MOE (group 3), HS+Vit. C (group 4), and HS+NaHCO<sub>3</sub> (group 5). HS groups were exposed to high temperatures (4 hours/day for 6 weeks), while treatments were given in drinking water during experiment period. Levels of blood cortisol and leptin, IL-1  $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and IL-10 were assayed using ELISA technique, while adrenaline was assayed calorimetrically. Expression of HSP70, FOXP3, T cell receptor (TCR)  $\gamma$  and  $\delta$ , lysozyme mRNA were tested using real-time (RT)-PCR, while HSP70 protein expression was tested using western blotting in liver and kidney tissues. Expression of lysozyme mRNA as well as activities of lysozyme, complement and antioxidants were assayed. Infiltration of NK (CD56<sup>+</sup>) cells, and regulatory T cells (Treg; CD25<sup>+</sup>) were tested using immunohistochemistry (IHC). Serum markers of liver damage (ALT & AST) and kidney damage (urea & creatinine) were assayed calorimetrically, while body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were calculated. Moreover, the histopathological changes of liver and kidney as well as mortality rate (MR) of experimental animals were investigated. Data showed increased levels of cortisol, adrenaline, leptin, IL-1  $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ , ALT, AST, urea and creatinine but decreased IL-10 in the HS group. Increased expression of HSP70 on both mRNA and protein levels was associated with increased NK and  $\gamma\delta$  T cells versus decreased Treg cell infiltration in liver and kidney tissues of the HS group. This is beside to decreased lysozyme and antioxidant activities versus increases in both complement activity and MR. In the same group, BWG was decreased, while FCR was increased with respect to the control group. Histological investigation showed an increase in inflammatory reactions. Whereas, all treatments used in this study could reverse HS-induced effects significantly and restore normal levels. In conclusion, MOE, Vit. C and NaHCO<sub>3</sub> can be added to rabbit diets for the relief of HS-induced symptoms.

Key words: Rabbit, heat stress, heat shock protein, immunology, *Moringa oleifera*, vitamin C, sodium bicarbonate