

Effects of Acute Exposure to Heat Stress on Immunological and Lipid Parameters in Rats

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Abstract

Background: Exposure to heat stress can result in lipid parameters alterations as well as immunological responses. This study was aimed to investigate these alterations and responses at different humidity and temperatures levels under controlled laboratory conditions in adult male Sprague–Dawley rats. **Methods:** A total 15 adult male Sprague–Dawley rats, weighing between 200 and 250 g, 10-11 weeks of age, were used in the evaluation. After one week, animals were randomly divided into three equal groups of 5 animals each. Reference group (group1) was housed in the cage under WBGT of 20(1) according to experimental design. Also, animals were subjected to mild (WBGT of 29 (1)) and hotter (WBGT of 33(1)) conditions (8 hr/day for one day) in the exposure chamber as group2 and group3, respectively. At the end of exposure to heat stress, blood samples were collected from the heart of rats and different parameters were determined. **Results:** Based on results, acute heat exposure significantly caused a decrease in serum IgG, IgM, IgA of rats in the case group compared to control group ($P<0.05$). Conversely, acute heat exposure resulted in a significant increase in the levels of IgE ($P<0.05$). There was no statistical difference for the lipid parameters after exposure to acute heat stress compared with the control group ($P > 0.05$). **Conclusion:** Acute heat stress may affect immune responses depending on the intensity of the exposure. Higher environmental temperatures ($WBGT\geq 32^{\circ}C$) cause more severe changes in plasma immunoglobulins.

Keywords: WBGT; Sprague–Dawley rats; Heat Stress; Immunological Parameter; Lipid Parameter

Introduction

The thermoregulatory system operates to maintain a steady body temperature under a variety of external conditions including changes in temperature, air velocity, and relative humidity fluctuations. The stress response is a very complex and multifaceted mechanism including a series of behavioral and hematological alterations as well as biochemical and immunological (Ig) responses by which one will be able to

meet the body's demands, adapt to them, and survive.¹⁻⁶ Therefore, it is necessary to protect humans and animals against health risks of heat stress in hot environments. For this purpose, the first step is identification of heat stress and related disorders. Although various studies have been conducted to assess the health effects of exposure to heat stress over the past years,¹⁻⁹ little information exists about the effects of heat stress on the plasma immunoglobulin.

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The hallmark of papers on stress and immune system generally refers to the occurrence of immunosuppression with subsequent failures in immune system activity development, particularly when the animals are challenged with vaccines, bacteria, pathogenic viruses, and other microorganisms.¹⁰⁻¹³

However, some studies have reported that exposure to high ambient temperature modifies various components of immune function such as T cell counts, natural killer (NK) cell counts, cytokine secretion, cytolytic activity, and lymphocyte proliferation.¹⁴ The observed changes in the lipid levels are important because of their influence on the risk of coronary heart disease (CHD) and other cardiovascular problems. High level of total cholesterol is a predictor of coronary heart disease mortality in humans.¹⁵ Elevated level of triglycerides and low-density lipoprotein (LDL) are known risk factors for atherosclerosis. And also a relationship between LDL and myocardial infarction among men has been recently established. In addition, low levels of high-density lipoprotein (HDL) have been found to be another independent risk factor for heart diseases. The probable relation between temperature and serum lipid level has rarely been investigated. High ambient temperature may depress de novo fatty acid synthesis in adipose tissues and in the liver.¹⁶ Changes in HDL and LDL levels with increasing of the ambient temperature may be mentioned as an underlying mechanism for temperature-related cardiovascular mortality.¹⁵

The measurements of changes in immunological and lipid parameters can be used to evaluate the physiological strain resulting from exposure to heat. So, changes in the levels of these parameters may be considered as the probable indicators for exposure to heat stress. Therefore, the present study was aimed to investigate the influence of acute heat stress on immunological parameters as well as lipid parameters at different humidity and temperatures

levels under controlled laboratory conditions in adult male Sprague–Dawley rats. The applicable aim of the study was introducing the appropriate indicators in order to help minimize stress in humans and animals.

Methods

This case control study was performed in the medical research center of Larestan, a city in South of Iran, in 2017.

Animals

A total 15 adult male Sprague–Dawley rats (weight of 200 to 250 g) were purchased from an animal's laboratory (Larestan, Iran). Animals were housed in a temperature (22 to 25 °C) and humidity (approximately 35-40%)-controlled colony room on a 12:12 h light: dark schedule (light on at 7 a.m . and light off at 7 p.m.) and were kept in School of Public Health and Animal Science, University of Larestan, according to environmental standards. To facilitate eating food, rats were housed conventionally in cages, with food and water provided ad libitum.

Exposure chamber

A rectangular chamber (35cm diameter, 40 cm height, and 80 cm length) was designed, constructed, and tested as a tool to measure the effects of heat stress on rats Figure 1. The chamber had a stainless steel (8-mil) frame covered with clear double glazing. The chamber design facilitated animal observation during exposures, provided proper air exchange, and resolved problems related to animal waste removal.¹⁷ Temperature and humidity were generated using a heater and humidifier (Hi-Tec HI-AH26, Canada) and measured in the chamber using a super digital timer (DTB-8MA, Iran) and two 800036 Wet Bulb Globe Temperature (WBGT) (Sper Scientific, china).

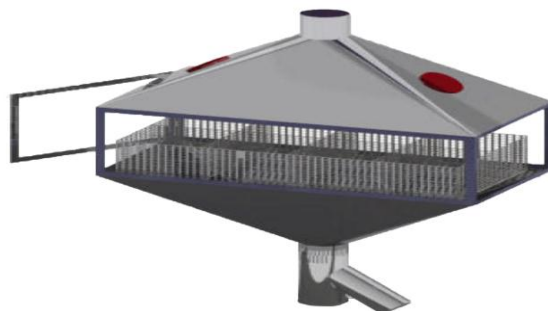


Figure 1. Exposure chamber

Exposure to heat stress

Three groups of adult male Sprague–Dawley rats (each group consist of 5 animals) were exposed to heat stress in an exposure chamber for 8 hours a day for one day as the acute exposure. After adaptation to the environmental changes, animals from each group were transferred to the exposure chamber. These animals were subjected to mild conditions in the chamber in which the WBGT was 29 (1) (dry bulb temperature of 32–34°C and relative humidity of 40–45%) as group2 (18). The experiments were repeated during the hotter mode (group3), that the mean WBGT was 33±1 (dry bulb temperature of 38–40°C and relative humidity 55–60%). Also, reference group (from the same strain) were housed in the cage under WBGT of 20(1) (dry bulb temperature of 22–25°C and relative humidity of 35–40%) as group1.

The subjects were exposed between 8:30 and 16:30. The WBGT in the chamber was recorded every half hour using two handheld WBGT devices (800036 Sper Scientific, china) simultaneously (for checking validity), and the mean of the obtained results was used. Also, heat and humidity in the chamber were measured and digitally controlled with(1)°C precision. The International Standard Organization (ISO) has proposed the WBGT-index for estimating the heat stress on workers in hot environments in the Standard ISO 7243.¹⁸

In the end of exposure to heat stress during one day (acute exposure), the rats were kept in cages to collect their blood.

Determination of blood parameters

Blood samples (4-5 mL) were collected from the heart of each rat into tubes and then centrifuged (3,000 rpm for 10 min) to obtain the serum. They were stored at -20°C (approximately 10 day) until analysis. A blood auto-analyzer of liquid phase (EasyVet, KONTROLab, Morelia, Mich., Mexico) was applied to determine plasma metabolite concentrations (cholesterol, triglyceride, HDL, LDL, VLDL, glucose). Furthermore, plasma immunoglobulins (IgG, IgA, IgE, and IgM) were determined by an automated analyzer (Cobas Mira; Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol.¹⁹

Data analysis

The statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). Some tests such as an independent T-test and a bivariate correlation were

used for data analyzing. The level of significance was taken as $p < 0.05$.

Ethics

The protocol of the present study was approved by the ethical committee of Shiraz University of Medical Sciences prior to the execution (Grant no. 1396-01-04-15122)

Results

The climatic conditions of the chamber have been displayed in Table 1. In thermal comfort conditions, the mean values of temperature, RH, and WBGT were 33(1)°C, 42.5 (2.5) %, and 29(1) °C, respectively. In the extreme heat stress, they were 39(1) °C, 57.5±2.5 %, and 33(1) °C, respectively. The WBGT values in hot mode indicated that rats in all groups were under heat stress conditions higher than ISO.^{17,18} The effect of heat stress on the changes in immune system and lipid parameters in adult male Sprague–Dawley rats can be seen in Table 2.

The mean body weights were 268(15.3)g, 265(14.1)g and 261(9.5)g, respectively, for the control, before and after exposure to acute heat stress. The exposure to acute heat stress did not induce significant changes in the body weights of rats ($P > .05$).

The comparison of the average values of serum immunoglobulins and lipid parameters between rats of case and control groups has been presented in Table 2. The results showed that the acute heat exposure to WBGT≥32°C significantly caused a decrease in serum IgG, IgM, IgA of rats in the case group compared to control group, but there was a significant increase in the levels of IgE ($P < 0.05$). There was no statistical difference for the lipid parameters after exposure to acute heat stress compared with the control group ($P > 0.05$).

The relationship between WBGT and immune system and lipid parameters was evaluated using a bivariate correlation Table 3. A moderate negative relationship was found between serum IgG and IgM parameters and the WBGT index in acute heat exposure. This relationship for IgG levels was negatively significant ($R_2 = -0.58$, $P < 0.05$). A direct correlation was reported between serum IgE levels and heat stress in acute exposure ($R_2 = 0.67$). Figures 2 and 3 (refer to Table 3 for information on correlation coefficient) show the relationship between WBGT and immunological and lipid parameters of Sprague–Dawley rats.

Table 1. Chamber climatic conditions recorded during the study (means(SD))

conditions	climatic parameters			
	Temperature(°C)	Relative humidity (%)	WBGT (°C)	ISO (°C)
Control	24 (1)	37.5 (2.5)	20 (1)	-
comfort	33 (1)	42.5 (2.5)	29(1)	28-30
Hot	39 (1)	57.5 (2.5)	33 9(1)	-

Table 2. Variation of immunological and lipid parameters of studied animals (mean (SD)) in different ranges of WBGT

Parameters	Control group	group 1	P-value	group 2	P-value
	(19-21 °C)	(28-31 °C)		(32-34 °C)	
IgG (g/l)	2.81 (0.67)	2.96 (0.13)	0.63	2.06 (0.05)	0.03
IgM (g/l)	0.45 (0.02)	0.51 (0.01)	0.003	0.28 (0.01)	0.001
IgA (g/l)	0.44 (0.04)	0.44 (0.05)	1	0.38 (0.01)	0.01
IgE (IU/ml)	3.38 (0.34)	3.74 (0.72)	0.34	5.92 (1.44)	0.005
TG(mg/dl)	77.20 (11.21)	65.20 (13.57)	0.16	73.60 (7.16)	0.56
CHOL(mg/dl)	79.80 (7.56)	80.60 (7.33)	0.86	63.40 (17.40)	0.08
HDL(mg/dl)	40.00 (4.69)	39.80 (5.06)	0.94	29.20 (9.39)	0.05
LDL(mg/dl)	24.40 (5.94)	24.60 (2.70)	0.94	18.80 (7.79)	0.23
VLDL(IU/l)	15.40 (2.07)	13.00 (2.73)	0.15	14.80 (1.48)	0.61

Statistical test , T- test; P<.05 considered statistically significant.

Table 3. Relationship between heat stress and immunological and lipid parameters (n=15)

Parameters	Correlation coefficient (R2)	P-value
IgG (g/l)	-0.65	0.008
IgM (g/l)	-0.58	0.02
IgA (g/l)	-0.51	0.05
IgE (IU/ml)	0.67	0.006
TG(mg/dl)	-0.20	0.46
CHOL(mg/dl)	-0.45	0.09
HDL(mg/dl)	-0.49	0.06
LDL(mg/dl)	-0.34	0.21
VLDL(IU/l)	-0.18	0.51

Statistical test: Pearson's test; the mean difference is significant at the .05 level.

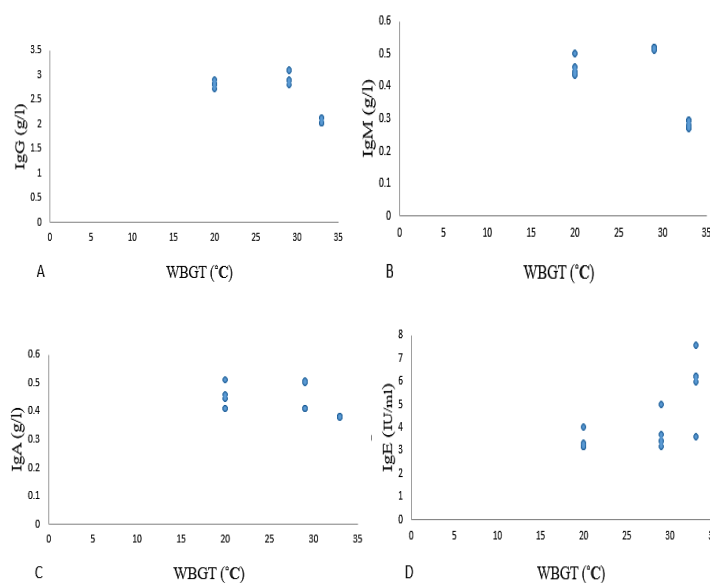


Figure 2. Scatterplot of WBGT and immunological and lipid parameters: correlation coefficient = -0.65, -0.58, -0.51 and 0.67, respectively, for the IgG, IgM, IgA and IgE.

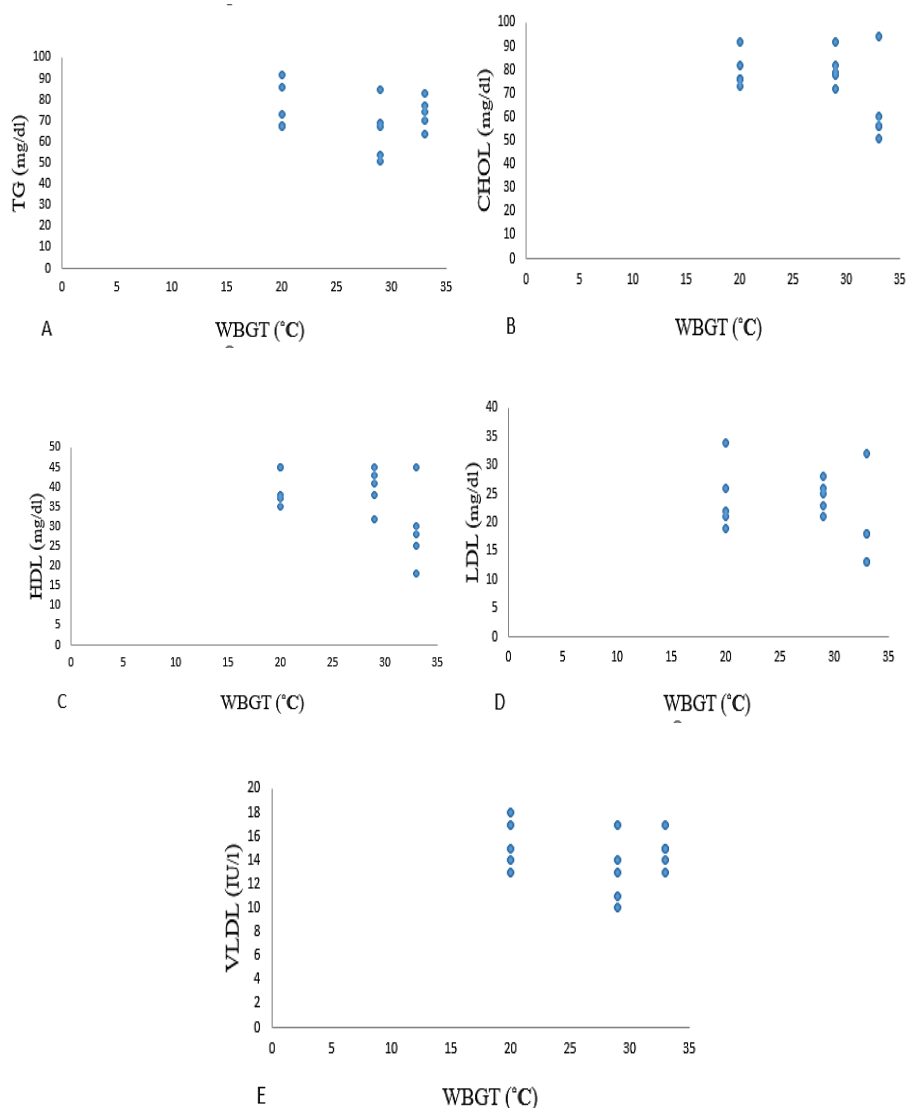


Figure 3. Scatterplot of concentrations of WBGT and immunological and lipid parameters: correlation coefficient = -0.2, -0.45, -0.49, -0.34 and -0.18, respectively, for the TG, CHOL, HDL, LDL and VLDL.

Discussion

In this study, the effect of vivo heat stress exposure on immunological and lipid parameters was investigated for adult male Sprague–Dawley rats. The observations indicated that the mean values of WBGT ($\geq 32^{\circ}\text{C}$) and the exposure duration (8 h) inside the chamber were sufficiently high to cause a state of heat stress. The rats showed obvious signs of heat exposure, such as rapid and shallow breathing, high body temperature, and a dispersed distribution within the chamber. The most abundant type of immunoglobulin in the blood is IgG (approximately 75% of serum antibodies) followed by smaller quantities of IgA and IgM. Therefore, most studies have focused on

heat stress-induced changes in serum IgA, IgG and IgM. The present study found that critical change in immunoglobulin values occurs when rats are exposed to heat stress. These findings are consistent with the data reported by Dhabhar et al. who noted that heat stress are able to suppress immunity.²⁰

The parameters of IgG, IgA, and IgM are often measured together. That way, they can give researchers important information about immune system functioning, especially relating to infection or autoimmune. This study also found the significant decreases in the levels of serum IgG, IgM, and IgA resulting from the acute exposure to heat. Evidence-based results show that heat stress creates a

greater threat to immune function in comparison with thermo-neutral conditions.^{21, 22} These findings are in line with the data reported by Niess et al. and Galloway et al., who suggest that the immune function may be negatively affected by exercise in extreme temperature compared to exercise in the thermo-neutral and cool condition.^{23, 24} In contrast, Muhamad et al. (2016) noted that salivary IgA concentration and secretion rate were not significantly different between exercise in the heat and cool condition which is in agreement with some previous studies.²⁵⁻²⁷ Although, comparisons cannot be made between similar serum antibodies of different species, according to Gomes et al. (2014), stressors are able to increase the immunoglobulin levels at least 24 h after application of the stress stimuli in broiler chickens.²⁸ It is worth mentioning that, the B-cell in bone marrow produce different immunoglobulins and birds' IgM has similar biological functions with that of mammals.²⁹ The difference between findings of mentioned studies can be associated with the age and type of species used or due to the experimental methodology applied. Regnier et al. suggested that heat-induced immunosuppression may depend on breed of bird or animal.³⁰ Kelley reported that the heat may affect the immune responses depending on the length and intensity of the heat exposure or the susceptibility of animals.³¹ According to Park et al, the decreased production of cells containing immunoglobulin and lowered levels of serum immunoglobulin can be related to the regression of the lymphoid organs under extreme heat stress.²⁹ A related point to consider in this study is that, the level of serum IgE (plays a pivotal role in reactions to allergens) increased in acute exposure to extreme heat. Thus, further researches should be performed to find the causes of these variations in the serum levels of IgE.

The serum lipid levels were lower in case group during their exposure to extreme heat stress. However, no significant decrease was detected in the levels of lipids during the acute exposure to extreme heat stress for one days. The main purpose of VLDL is to carry the triglyceride synthesized in the liver. Omran et al. and Pandey et al. also reported significant decreasing of triglyceride levels during heat stress in Egyptian buffalo calves and Marwari goats, respectively.^{32, 33} This reduction can be attributed to the increase of hormone sensitive TG-lipase as a result of enhanced release of the cortisol during

heat stress.³⁴ Base on mentioned results, the disturbance of plasma lipids is closely related to the "intensity" of heat challenge during heat exposure. Higher environmental temperatures, up to WBGT \geq 32°C cause more severe changes in plasma lipids.

Conclusion

This study investigated alterations of immunological parameters as well as lipid responses at different humidity and temperatures levels in adult male Sprague–Dawley rats. In acute exposure to heat stress the alteration of some parameters was considerable and there was a relationship between them and the WBGT index. It is worth mentioning that higher environmental temperatures (WBGT \geq 32°C) can cause more severe changes in immunological parameters of the serum. Therefore, further study should be taken to study the immune system and metabolic parameters in humans when they are exposed to heat stress.

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References

1. Haque N, Ludri A, Hossain SA, Ashutosh M. Impact on hematological parameters in young and adult Murrah buffaloes exposed to acute heat stress. *Buffalo Bull.* 2013;32(4):321-6.
2. Shini S, Huff GR, Shini A, Kaiser P. Understanding stress-induced immunosuppression: Exploration of cytokine and chemokine gene profiles in chicken peripheral leukocytes 1. *Poultry science.* 2010;89(4):841-51.
3. Bourbonnais R, Zayed J, Levesque M, Busque M-A, Duguay P, Truchon G. Identification of Workers Exposed Concomitantly to Heat Stress and Chemicals. *Industrial Health.* 2013;51(1): 25-33.
4. Crowe J, Wesseling C, Solano BR, Umaña MP, Ramirez AR, Kjellstrom T, Morales D, Nilsson M. Heat exposure in sugarcane harvesters in Costa Rica. *American Journal of Industrial Medicine.* 2013;56(10):1157-64.
5. Dunne JP, Stouffer RJ, John JG. Reductions in labour capacity from heat stress under climate warming. *Nature Climate Change.* 2013;3(6):563.
6. Monazzam, Mohamadreza, et al. "Evaluation of DI, WBGT, and SWreq/PHS heat stress indices for estimating the heat load on the employees of a petrochemical industry." *International Journal of Occupational Hygiene* 6.1 (2015): 6-10.
7. Honda BTB, Calefi AS, Costola-de-Souza C, Quinteiro-Filho WM, da Silva Fonseca JG, de Paula VF, Palermo-Neto J. Effects of heat stress on peripheral T and B lymphocyte profiles and IgG and

- IgM serum levels in broiler chickens vaccinated for Newcastle disease virus. *Poultry science*. 2015;94(10):2375-81.
8. Waltz X, Baillot M, Connes P, Bocage B, Renaudeau D. Effects of hydration level and heat stress on thermoregulatory responses, hematological and blood rheological properties in growing pigs. *PLoS one*. 2014;9(7):e102537.
 9. Calefi AS, Honda BTB, Costola-de-Souza C, de Siqueira A, Namazu LB, Quinteiro-Filho WM, et al. Effects of long-term heat stress in an experimental model of avian necrotic enteritis. *Poultry science*. 2014;93(6):1344-53.
 10. Kokaia Z, Martino G, Schwartz M, Lindvall O. Cross-talk between neural stem cells and immune cells: the key to better brain repair [quest]. *Nature neuroscience*. 2012; 15(8):1078.
 11. Kaiser P. Advances in avian immunology-prospects for disease control: a review. *Avian Pathology*. 2010;39(5):309-24.
 12. Costa-Pinto FA, Palermo-Neto J. Neuroimmune interactions in stress. *Neuroimmunomodulation*. 2010;17(3):196-9.
 13. Nasrin M, Khan MZI, Siddiqi MNH, Masum MA. Mobilization of immunoglobulin (Ig)-containing plasma cells in Harderian gland, cecal tonsil and trachea of broilers vaccinated with Newcastle disease vaccine. *Tissue and Cell*. 2013;45(3):191-7.
 14. Shephard RJ. Immune changes induced by exercise in an adverse environment. *Canadian journal of physiology and pharmacology*. 1998;76(5):539-46.
 15. Halonen JI, Zanobetti A, Sparrow D, Vokonas PS, Schwartz J. Outdoor temperature is associated with serum HDL and LDL. *Environmental research*. 2011;111(2):281-7.
 16. Xin WU, LI Z-Y, JIA A-F, SU H-G, HU C-H, Zhang M-H, et al. Effects of high ambient temperature on lipid metabolism in finishing pigs. *Integrative Agriculture*. 2016;15(2):391-6.
 17. Wong BA. Inhalation exposure systems: design, methods and operation. *Toxicologic Pathology*. 2007;35(1):3-14.
 18. ISO. Hot environments-estimation of the heat stress on working man, based on the WBGT index (web bulb globe temperature). 2nd ed. Us: ISO7243; 1989. P:9.
 19. Zamanian Z, Yousefinejad S, Khoshnoud MJ, Golbabaie F, Farhang Dehghan S, Modaresi A, et al. Toxic effects of subacute inhalation exposure to trichloroethylene on serum lipid profile, glucose and biochemical parameters in Sprague-Dawley rats. *Inhalation toxicology*. 2018;30(9-10):354-60.
 20. Dhabhar FS, Saul AN, Daugherty C, Holmes TH, Bouley DM, Oberyszyn TM. Short-term stress enhances cellular immunity and increases early resistance to squamous cell carcinoma. *Brain, behavior, and immunity*. 2010;24(1):127-37.
 21. Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, et al. Position statement part two: maintaining immune health. Loughborough University Institutional Repository. 2011;17:64-103
 22. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop N, et al. Position statement part one: immune function and exercise. Loughborough University
 23. Institutional Repository 2011; 17:6-63.
 24. Niess AM, Fehrenbach E, Lehmann R, Opavsky L, Jesse M, Northoff H, et al. Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise. *European journal of applied physiology*. 2003;89(3-4): 344-51.
 25. Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Medicine and science in sports and exercise*. 1997;29(9):1240-9.
 26. Muhamad AS, Chen CK, Ayub A, Ibrahim NS. Effects of Prolonged Exercise in the Heat and Cool Environments on Salivary Immunoglobulin A among Recreational Athletes. *Sports and Physical Education*. 2016;3(4):51-6
 27. Laing SJ, Blackwell J, Gwynne D, Walters R, Walsh NP. Neutrophil degranulation response to 2 hours of exercise in a 30 degrees C environment. *Aviation, space, and environmental medicine*. 2005;76(11):1068-73.
 28. Akimoto T, Kim K, Yamauchi R, Izawa S, Hong C, Aizawa K, et al. Exercise in, and adaptations to a cold environment have no effect on SIgA. *Sports medicine and physical fitness*. 2009;49(3):315-319.
 29. Gomes, AVS, Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Baskeville E, et al. Overcrowding stress decreases macrophage activity and increases Salmonella Enteritidis invasion in broiler chickens. *Avian pathology*. 2014;43(1): 82-90.
 30. Park S-O, Hwangbo J, Ryu C-M, Park B-S, Chae H-S, Choi H-C, et al. Effects of Extreme Heat Stress on Growth Performance, Lymphoid Organ, IgG and Cecum Microflora of Broiler Chickens. *International Journal of Agriculture & Biology*. 2013;15(6).
 31. Regnier JA, Kelley KW, Gaskins CT. Acute Thermal Stressors and Synthesis of Antibodies in Chickens. *Poultry Science*. 1980;59(5):985-90.
 32. Kelley KW. Immunobiology of domestic animals as affected by hot and cold weather. *Transactions of the ASAE*. 1983; 26(3): 834-40.
 33. Omran FI, Ashour G, Youssef MM, Shafie MM. Responses of hematology, blood metabolites, mineral ions and hormonal profile to heat stress for Egyptian buffalo-calves. *Egypt. J. Agric. Res*. 2011;89(3):1129-38.
 34. Pandey N, Kataria N, Kataria AK, Joshi A. Ambient stress associated variations in metabolic responses of Marwari goat of arid tracts in India. *Stress Physiology & Biochemistry*. 2012; 8(3).