# STUDY CONCERNING THE THERMAL STRESS IMPACT ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN DOGS

Bianca Gabriela HAGIU, Iuliana CODREANU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei Street, District 5, Bucharest, Romania

Corresponding author email: biancagabrielah26@gmail.com

#### Abstract

The environmental temperature exceeding either the inferior or the superior limit of the thermo-neutrality zone may lead to thermal stress, due to the cold or the heat. This study was conducted in order to determine the changes in hematological and biochemical parameters of dogs exposed to thermal stress. A total of 20 dogs were randomly divided into two groups (10 per group). Group 1 (control group) was exposed to an environmental temperature of  $15\pm1^{\circ}$ C, while the second group to  $40\pm2^{\circ}$ C, to induce thermal stress caused by the heat. Blood samples were collected and subjected to investigations. This study showed that the blood sugar level significantly increased in group 2. However, PCV, Hb, and also cholesterol, triglycerides, total protein, and albumin levels were significantly decreased in the experimental group. At the same time, MCH, MCHC, WBC and the globulin levels did not show any significant changes from the control group. It was concluded that thermal stress in dogs could negatively affect the mechanism of thermal regulation in dissipating excess body temperature, so their general condition may change considerably.

Key words: biochemical parameters, dog, heat, hematological parameters, thermal stress.

# **INTRODUCTION**

During the last 50-100 years there was a tendency for climate extremes in many parts of the world (Heim, 2015). Global climate change represents one of the most significant challenge that is threatening public health; both antropogenic (industrial activities) and natural factors (volcanoes, solar variations) are increasing the temperature and the amount of precipitations (Easterling et al., 2016). It was reported that the world is experiencing fewer cold days and nights. This trend is due to the increasing number of warm days and nights (Heim, 2015). Animals have a range of functional systems controlling body temperature, nutritional state, social interaction which allow the individuals to control its interactions with its environment and hence to keep each aspect of its state within a tolerable range (Broom, 1996). Maintaining the internal temperature within certain boundaries, even if the surrounding temperature is very different, is a process called thermoregulation (Codreanu, 2018). Heat balance occurs through the actions of heat gain and dissipation mechanism (Mazzaferro, 2009). Besides the growth, milk production, pregnancy and activity components, the exchange of heat between animals and the environment is also an important component of metabolic heat production (Berman, 2003). The environmental temperature exceeding either the inferior or superior limit of the therm-neutrality zone may lead to thermal stress, due to the cold or the heat (Cotor et al., 2014). Each species has optimal temperature limits, allowing the physiological processes to run properly; otherwise, it appears the stressful state (Barton, 2002; Cocan et al., 2018). The temperature can affect the growth rate, feed efficiency, animal behaviour and also reproductive efficiency (Bogdan, 1999; Vasile et al., 2012). The sensitive and psychic stress agents can hurt the animals and degrade their welfare. In this situation, the physiological, metabolic, and hematologic parameters are modified and the deterioration of the animal welfare degree might be valued (Paraschivescu & Paraschivescu, 2012). Dogs are part of the category of domestic animals used by humans for thousands of years in their lives for many purposes, such as hunting, detecting drugs, searching for missing people, guarding, or guiding blind people (Culea et al., 1998). Even if the average temperature of dogs is 37.9-38.9°C and they regulate it by the nervous system, the thermal

stress is very common in dogs (Cunningham, 1992). Multivariable analysis identified significant risk factors including geriatric age, sex, breed (brachycephalic anatomy), obesity, an active playful character (Labrador and Golden retrievers), utility (military and police working dogs) (Bruchim et al., 2017; Hall et al., 2020; Niedermeyer et al., 2020; Moon et al., 2021). Results from studies conducted in other species revealed that the lack of hair and its density caused a predisposition to thermal stress (Araúz, 2017; Pena et al., 2020). Also, heat dissipation can be affected by the use of certain drugs (diuretics, phenothiazines, negative inotropic drugs) (Romanucci & Della Salda, 2013). This study was realized to determine the changes in hematological and biochemical parameters of dogs exposed to thermal stress.

## MATERIALS AND METHODS

This study was conducted in August 2020, when it was a prolonged hot period. A total of 20 clinically healthy dogs (females and males), raging between 1-3 years old and with approximately equal body weights were used. The dogs were randomly divided into two groups, with 10 dogs per group. They were placed into a room with AC installation. The dogs from group 1 (control group) were exposed to an environmental temperature of  $15\pm1^{\circ}$ C, while the group 2 to a higher temperature, to induce thermal stress caused by the heat. For this to be done, the dogs from the second group were exposed to an environmental temperature of  $40\pm2^{\circ}$ C for 3 h/day, 7 days.

Blood samples were collected by puncturing the cephalic vein puncture and subjected for investigations. The samples were transferred into 2 ml EDTA vacutainers (for hematological tests) and into 3 ml Clot activator vacutainers (for the biochemical profile) and transported under refrigeration conditions  $(+2^{\circ}C)$  to the laboratory.

The hematological parameters that were taken under consideration were: red blood cells, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells. The following biochemical parameters were investigated: blood sugar, total protein, albumin, globulin, cholesterol and triglycerides. Hematological parameters were determined by using IDEXX VetAutoreadTM Hematology Analyzer. Serum biochemistry parameters' analysis was performed with IDEXX VetTest 8008. The obtained results were compared with the reference values and for the statistical interpretation of the obtained data was performed Student's t-test. The data are expressed as means  $\pm$  SD. Differences were compared for statistical significance at the p-level less than 0.05 (P<0.05). Tables and charts were designed in Word and Excel, Microsoft Office 2010.

# **RESULTS AND DISCUSSIONS**

The hematological analyses presented in Table 1 showed significant decreases (p<0.05) of packed cell volume (PCV) and hemoglobin (Hb) in group 2, in comparison to group 1. However, the red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cells (WBC) did not show significant changes (p>0.05) in group 2 from the control group (Figure 1).

Table 1. Hematological data of dogs from group 1 and group 2

Hematological parameters	Group 1	Group 2
RBC (1×10 <sup>6</sup> /mm <sup>3</sup> )	6.32±0.28	5.25±0.23*
Hb (g/dl)	14.87±0.27	10.13±0.27**↓
PCV (%)	43.75±0.33	32.97±0.33**↓
MCV (µ <sup>3</sup> )	69.31±2.69	62.41±2.83*
MCH (pg)	23.52±0.80	19.27±0.48*
MCHC (%)	34.04±0.63	30.69±0.73*
WBC (1×10 <sup>3</sup> /mm <sup>3</sup> )	9.68±0.16	10.13±0.15*

(Mean ± Standard Deviation)

\*p>0.05 - statistically non-significant differences;

\*\*p<0.05 - statistically significant differences



Figure 1. Variation of hematological parameters of dogs in group 1 and group 2

The biochemical profile and the effect of different environmental temperatures on it are presented in Table 2, Figure 2a, and Figure 2b. The blood sugar level increased significantly (p<0.05) in group 2 from the first group. On the other side, the levels of total protein, albumin, cholesterol and triglycerides showed significant decreases (p<0.05) in group 2 from the control group. The globulin level of group 2 shows a statistically non-significant increase (p>0.05) from group 1.

Table 2. Biochemical data of dogs from group 1 and group 2

Biochemical parameters	Group 1	Group 2
Total protein (g/dl)	6.12±0.23	4.92±0.35**↓
Albumin (g/dl)	3.91±0.23	1.97±0.17**↓
Globulins (g/dl)	2.21±0.14	2.95±0.21*
Blood sugar (mg/dl)	82.17±0.23	96.1±0.39**↑
Cholesterol (mg/dl)	143.25±0.49	90.38±0.37**↓
Triglycerides (mg/dl)	61.9±0.37	35.6±0.63**↓

(Mean ± Standard Deviation)

\*p>0.05 - statistically non-significant differences;

\*\*p<0.05 - statistically significant differences



Figure 2a. Variation of proteic profile of dogs in group 1 and group 2



Figure 2b. Variation of energetic profile of dogs in group 1 and group 2

Blood is the most efficient stress indicator (Hattingh, 1977; Marin et al., 2015; Simide et al., 2016). The environment may have a significant impact upon the hematological parameters in mammalian and non-mammalian vertebrates (Gabriel et al., 2004).

The caloric needs of working or stressed dogs may exceed the levels of a maintenance diet, depending on the animal and the extent of work performed. Most diets designed for work or stress have increased levels of animal fats, with the other nutrients appropriately balanced to the increased energy density (The Merck Veterinary Manual). Hyperthermia leads to an metabolic state increased and oxvgen consumption that raise both caloric and water requirements by approximately 7% for each 0,6°C above accepted normal values (Miller, 2009). In addition, hyperthermia leads to suppression of the appetite center in the hypothalamus, causing a decrease in feed consumption (Miller, 2009; Wojtas et al., 2014). Studies conducted by Taha (1998) and Alkam (1999) explained that exposure of dogs (group 2) to high environmental temperature led to malabsorption of essential elements for red blood cells formation like iron and cobalt. The reduction of packed cell volume (PCV) and hemoglobin (Hb) could be considered as a result of RBC reduction (Al-Shammari et al., 2019).

Regarding the protein profile, the significant decrease in the average concentrations of total blood protein and albumin levels in group 2 can be due to the thermal injury and increased cellular metabolic demand and oxygen consumption; the thermal injury may cause widespread cellular necrosis through protein denaturation (Flournoy et al., 2003).

According to Patriche et al. (2011) and Suljević et al. (2015), a stress indicator valuable biomarker is the level of glycemia. The significant decreases in cholesterol and triglycerides levels observed in group 2 may be resulted from the lower metabolic rate, due to the lower thyroxin (T4) concentration, as Chandra et al. (2009) observed.

#### CONCLUSIONS

It was concluded that exposing dogs to an environmental temperature of  $40\pm2^{\circ}$  C leads to

an increased metabolic state and oxygen consumption.

The physiological response to the thermal stress factors is manifested by significant variations of cortisol and blood glucose.

The glycemia in dogs exposed to an environmental temperature of  $40\pm2^{\circ}$ C (for 3 h/day, 7 days) was significantly increased in thermal stress conditions, explained by highly increased cortisol secretion by the adrenal gland.

### REFERENCES

- Al-Akam, Ali A (1999). Effect of varying temperature levels in dogs exposed to stress. J. King Faisal Uni. Agric. Sci. 5(11): 72–64.
- AL-Shammari, A., Al- Hashimi, A.D., & Rabeea, A.H. (2019). Impact of Thermal Stress on Health Signs, Hormones Levels, Hematological and Biochemical Parameters of Police Dogs in Iraq. *Advances in Animal and Veterinary Sciences.*
- Araúz E. (2017). Influence of fur color on body thermal behavior, kinetics of caloric overload and circadian cardiorespiratory alteration in crossed dairy cows (6/8 Bostaurus x 2/8 Bosindicus) under heat stress in the humid tropics. *Electronic Veterinary Magazine* -ISSN 1695–7504. Volume 18 No. 7.
- Barton B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42(3): p. 517–525.
- Berman A. (2003). Effects of body surface area estimates on predicted energy requirements and heat stress. *Journal for Dairy Science*. 86: 3605–3610.
- Bogdan A.T., Mantea S. T., Bogdan D. (1999). Tratat de reproducție şi însămânțări artificiale la suine. Bucharest, RO: Editura Tehnică Agricolă.
- Broom D. M. (1996). Animal welfare defined in term of attempts to cope with the environment. *Acta Agric. Scand. Sect. A. Animal Sci.* Supplementum, no. 27, p. 22–28.
- Bruchim Y., Klement E., Saragusty J., Finkeilstein E., Kass P., Aroch I. (2006). Heat stroke in dogs: A retrospective study of 54 cases (1999-2004) and analysis of risk factors for death. *J Vet Intern Med.* 20(1):38–46.
- Chandra N. O. (2009). The growing problem of obesity in dogs and cats. J. Nutr. 136 (suppl.):1940S–6S.
- Cocan, D., Popescu, F., Latiu, C., Uiuiu, P., Coroian, A., Raducu, C., Coroian C.O., Miresan, V., Kokkinakis, A., & Constantinescu, R. (2018). Effects of thermal stress on hematological and metabolic profiles in Brown bullhead, Ameiurus nebulosus (LESUEUR, 1819). AgroLife Scientific Journal, 7(1), 33–41.
- Codreanu I. (2018). *Textbook of Animal Physiology*. Bucharest, RO: Editura Printech.
- Cotor G., Ghiță M., Bălăceanu R.. (2014). Note de curs și lucrări practice de fiziopatologie generală. Bucharest, RO: Editura Printech.

- Culea C., Nicolae M., Petroman I. (1998). Creșterea câinilor. Timișoara, RO: Editura Mirton
- Cunningham J. G. (1992). Textbook of veterinary physiology. USA: W. B. Saunders Company.
- Easterling D, Kunkel K, Wehner M, and Sun L. (2016). Detection and attribution of climate extremes in the observed record. *Weather Clim. Extrem.* 11: 17–27.
- Flournoy S. W., Wohl J. S., Macintire D. K. (2003). Heatstroke in dogs: pathophysiology and predisposing factors. *Comp Cont Edu Pract Vet* 2003;25:410–418.
- Gabriel U.U., Ezeri G.N.O., Opabunmi O.O. (2004). Influence of sex, source, health status and acclimation on the haematology of Clarias gariepinus (Burch,1822). *African Journal of Biotechnology*, 3(9), 463–467.
- Hattingh J. (1977). Blood sugar as an indicator of stress in the freshwater fish, *Labeo capensis* (Smith). *Journal of Fish Biology*, 10(2): p. 191–195.
- Hall E. J., Carter A. J., Bradbury J., Barfield D., O'Neill D. G. (2021). Proposing the VetCompass clinical grading tool for heat-related illness in dogs. *Sci Rep.* 11(1): 6828.
- Hall E. J., Carter A. J., O'Neill D. (2020) G. Dogs Don't Die Just in Hot Cars-Exertional Heat-Related Illness (Heatstroke) Is a Greater Threat to UK Dogs. *Animals (Basel)*. 10(8):1324.
- Hall E. J., Carter A. J., O'Neill D. G. (2016). Incidence and risk factors for heat-related illness (heatstroke) in UK dogs under primary veterinary care in 2016. *Sci Rep.* 10(1):9128.
- Heim Jr R. (2015). An overview of weather and climate extremes - Products and trends. *Weather Clim. Extrem.* 10, Part B: 1–9.
- Marin M., Nicolae C., Drăgotoiu D., Urdeş L., Răducuţă I., Diniţă G. (2015). Researches regarding the haematological profile of juvenile Cyprinus carpio varieties. *Scientific Papers. Series D. Animal Science*, Vol. LVIII, p. 209–212.
- Martínez-Porchas M., Martínez-Córdova L.F., Ramos-Enriquez R. (2009). Cortisol and glucose: Reliable indicators of fish stress? *Pan-American Journal of Aquatic Sciences*, 4(2): p. 158–178.
- Mazzaferro E. M. (2009). Heatstroke.In Ettinger S. J., Feldman E. C. (Ed), *Textbook of Veterinary Internal Medicine* (p 509–512). USA: Elsevier Health Sciences.
- Miller J. B. (2009). Hyperthermia and fever of unknown origin. In Ettinger S. J., Feldman E. C. (Ed), *Textbook* of Veterinary Internal Medicine (p 41–47). USA: Elsevier Health Sciences.
- Moon K. E., Wang S., Bryant K., Gohlke J. M. (2021). Environmental Heat Exposure Among Pet Dogs in Rural and Urban Settings in the Southern United States. *Frontiers in Veterinary Science*. 8:742926.
- Niedermeyer, G. M., Hare, E., Brunker, L. K., Berk, R. A., Kelsey, K. M., Darling, T. A., Nord, J. L., Schmidt, K. K., & Otto, C. M. (2020). A Randomized Cross-Over Field Study of Pre-Hydration Strategies in Dogs Tracking in Hot Environments. *Frontiers In Veterinary Science*, 7, 292.
- Paraschivescu, M.T., & Paraschivescu, M.T. (2012). Psychic stress and animal welfare in dairy cattle

production. Scientific Works - University of Agronomical Sciences and Veterinary Medicine, Bucharest. Series C, Veterinary Medicine, 58, 187–196.

- Patriche T., Patriche N., Bocioc E. (2011). Determination of some normal parameters in juvenile Sevruga sturgeons Acipenser stellatus (Pallas, 1771). Archiva Zootechnica, 14(1): p. 49–54.
- Pena I. G., Vidal F. F., Pedraza R. O., Arnaldo del Toro R., Hernández A. R. (2020). Effect of Thermal Stress on Physiological Parameters in Dogs from the Tropical Region of Camagüey, Cuba. Archives of Veterinary and Animal Sciences 2(1).
- Romanucci, M., & Della Salda, L. (2013). Pathophysiology and pathological findings of heatstroke in dogs. *Veterinary Medicine: Research and Reports*, 1.
- Simide R., Richard S., Prévot-D'Alvise N., Miard T., Gaillard S. (2016). Assessment of the accuracy of physiological blood indicators for the evaluation of stress, health status and welfare in Siberian sturgeon (Acipenser baerii) subject to chronic heat stress and

dietary supplementation. *International Aquatic Research*, 8(2): p. 121–135.

- Suljević D., Islamagić E., Fočak M., 2015. The effect of high temperature level on electrolytes and glucose concentration in tench (*Tinca tinca* Linnaeus, 1758) serum. Veterinaria, 64(2): p. 60–64.
- Taha A. R. J. (1998). Effect of thermal stress on the qualitative traits of puppies in dogs. PhD thesis, College of Agriculture. Cairo University.
- The Merck veterinary manual. Whitehouse Station, NJ :Merck & Co., Inc.
- Vasile, L., Şonea, A., Barţoiu, A.I., Radoi, I.E., & Posea, C. (2012). Influence of high temperature on reproduction in sows. Scientific Works - University of Agronomical Sciences and Veterinary Medicine, Bucharest. Series C, Veterinary Medicine, 58, 387– 391.
- Wojtas K., Cwynar P., Kołacz R. (2014). Effect of thermal stress on physiological and blood parameters in merino sheep. Bullet. *Vet. Inst. Pulawy.* 58(2): 283–288.