



The Effects of Weather Conditions on Hematological and Biochemical Parameters in Dogs: A Field Study

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ABSTRACT

Although animals have adaptation abilities to different environmental conditions, various physiological changes may occur. The present research aimed to evaluate the effects of severe winter conditions on hematological and biochemical parameters in dogs kept outside all year. The research was carried out in the province of Kars, which is known for its severe cold conditions in Türkiye. Vital signs, hematological, and biochemical parameters of 12 adult dogs aged 1-8 years old (mixed breed, 8 males and 4 females) included in the study were compared in winter and summer seasons. The results indicated a significant effect of the winter season on the body temperature, respiration, and pulse rate of the dogs. In addition, it was observed that some hematological, including White blood cell (WBC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) Hemoglobin (HB), and biochemical parameters (urea, TP, albumin, cholesterol, glucose, creatinine) of dogs differed in winter from those in summer. It should be considered that the vital values of dogs living outdoors in intense winter differed from those in mild winter or summer. It is also concluded that veterinarians should consider these differences in routine clinical examinations of these animals.

Keywords: Biochemical parameter, Cold stress, Dog, Environmental factor, Hematological parameter, Winter condition

INTRODUCTION

Dogs have been utilized in various fields due to the close relationship with humans that began thousands of years ago, being preferred for purposes ranging from search and rescue operations to hunting and protection duties (Al-Shammari et al., 2019). These days, dogs are meticulously monitored by their owners or breeders in matters, such as health check-ups, routine vaccination tracking, and treatment management. It's crucial to combine medical history, physical examinations, vital signs, and laboratory test outcomes when assessing the health status of animals (Erkiñiç, 2023). Blood analyses, particularly, play a critical role in assessing the health status of animals (Aryıybi et al., 2002), monitoring response to treatment, and detecting potential diseases (Erkiñiç, 2023). Valuable information about the general health status of the animals could be reached through blood parameters (Çınar et al., 2010). It can change under the influence of various factors (Mohammed et al., 2017), including environmental factors, such as age, gender, pregnancy, nutrition, habitat, and climate (Aryıybi et al., 2002; Erkiñiç, 2023).

Climate change is a major global concern that presents itself in a variety of ways. These manifestations include fluctuations in weather patterns, such as variations in annual precipitation and shifts in temperature (Serrano et al., 2021). Although animals have adaptive capabilities to cope with these climate changes, it's acknowledged that fluctuations in environmental temperature can impact their physiological responses (Ji et al., 2021). Severe winter weather conditions, characterized by low temperatures, can induce a range of physiological alterations in animals (Ji et al., 2021). Exceeding the lower or upper limit of the thermo-neutrality zone due to cold or heat can lead to thermal stress (Hagiu and Codreanu, 2022). Dogs are susceptible to the potential dangers of thermal stress as well (Al-Shammari et al., 2019).

Cold or thermal stress primarily increases the risk of hypothermia or freezing in dogs. Ji et al. (2021) reported it can also lead to a weakened immune system and a loss of resistance to infections and other diseases, which can be a direct cause or trigger, in combination with other factors, of many clinical problems. In areas characterized by severe winter, dogs serve as invaluable assistants to cattle and sheep farmers. These animals are typically kept outside, such as in a yard, and used for protective responsibilities. However, it is known that environmental factors, especially climate and temperature changes, can affect the health status of dogs.

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The present study aimed to determine the impact of severe winter conditions on dogs living continuously outdoors. To achieve this goal, it was planned to analyze the hematological and some routine biochemical parameters of the winter season and compare them with the values of the summer season.

MATERIALS AND METHODS

Ethical approval

The study was conducted after obtaining approval from the Kafkas University Animal Experiments Local Ethics Committee, Türkiye (KAU-HADYEK/2021-017).

Study location

Kars is located in the geographical coordinates of 42°10' and 44°49' East meridians, and 39°22' and 41°37' North parallels in the Northeast Region of Türkiye. The Kars region experiences significant variations in temperature between the summer and winter seasons (Şeker, 2001).

Meteorological data

Five-year meteorological data, including relative humidity, temperature, and rainfall amount for the region where the study was conducted, between the years 2017-2021, were obtained from the Kars Meteorology Directorate.

Animals

A total of 12 healthy adult dogs aged between 1 and 8 years (mix breed, 8 males and 4 females) were chosen randomly. The samples were kept continuously outdoors in the central district of Kars province, with regular antiparasitic and vaccination applications, under similar care and feeding conditions. The vital values and blood samples of the animals subjected to the experiment were obtained by going to the environment where the animals were living. According to the information from the animal owners, the animals were fed mostly home-made meals.

Blood sample

Blood samples were taken from the dogs twice, including in January, the coldest month, and in July, in the summer. An amount of ten mL blood samples was drawn from the *v. cephalica antebraehii* and placed into vacuum gel serum tubes (BD Vacutainer®, BD, UK) and vacuum EDTA blood tubes (BD Vacutainer®, BD, UK). Vacuum serum tubes were centrifuged at 3000 rpm for 10 minutes Hettich Rotina 380R®, Hettich, Germany, to obtain serum.

Vital parameters and hematological-biochemical analyses

Initially, clinical examinations were conducted, while the animals were at rest. The rectal body temperature (°C), pulse rate, and respiratory rate per minute were recorded. Hematological analyses were performed using a blood count analyzer (VG-MS4e®, Melet Schloesing, France) with samples collected in EDTA tubes. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatine kinase (CK) enzyme activities from the serum samples obtained were measured using the Mindray BS120® fully automatic biochemistry device (Mindray Medikal Technology). Cholesterol, triglyceride, glucose, creatinine, urea, total protein, and albumin levels were determined by the colorimetric method (Epoch, Biotek, USA) using commercial test kits (Biolabo, France). Globulin levels were obtained by subtracting albumin levels from total protein levels.

Statistical analysis

The statistical analysis of the obtained data was conducted using the SPSS version 25 software. The Shapiro-Wilk test was applied to determine whether the data in the groups were normally distributed. Based on the obtained results, the Paired Sample T-test was performed for normally distributed data, and the Wilcoxon test was used for non-normally distributed data. P-value < 0.05 was considered statistically significant.

RESULTS

The average meteorological data between 2017 and 2021 for the Central District of Kars Province is presented in Table 1. Upon examining the temperature averages spanning five years, it is observed that January, the month of sampling, had the lowest temperature, while July had the highest temperature. Accordingly, during January in which the study was conducted, the average temperature was recorded as -8.4°C, while the average temperature for July was 19.5°C.

Significant differences were found between vital signs evaluated in winter and summer ($p < 0.05$, Table 1). The average vital signs in the winter were as follows, body temperature was recorded as $38.21 \pm 0.17^\circ\text{C}$, respiratory rate was 29.83 ± 1.99 breaths/minute, and heart rate was 92.67 ± 3.22 beats/minute (Table 2). In the summer, the average of the same parameters was determined as follows, body temperature was $38.8 \pm 0.21^\circ\text{C}$, respiratory rate was 44.17 ± 5.70 breaths/minute, and heart rate was 111.00 ± 6.70 beats/minute.

As can be seen in Table 3, the hematological values obtained after the whole blood count conducted in January and July are presented. It has been observed that the total leukocyte count ($p = 0.034$) along with the mean corpuscular hemoglobin (MCH, $p = 0.005$), mean corpuscular hemoglobin concentration (MCHC, $p = 0.002$), and hemoglobin (Hb) concentration ($p = 0.006$) were statistically higher in the winter compared to the summer (Table 3).

Some biochemical parameters were analyzed in the serum obtained from blood samples taken in winter and summer and are presented in Table 4. Among the evaluated biochemical parameters, blood urea ($p = 0.027$), total protein ($p = 0.03$), albumin ($p = 0.022$), and creatinine levels were determined to be statistically significantly lower in winter than in summer ($p < 0.001$, Table 4). However, it was noted that the levels of glucose and cholesterol ($p = 0.021$) were significantly higher in the winter compared to the summer ($p < 0.001$, Table 4).

Table 1. Average of meteorological data for Kars Province, Central District, Türkiye, during 2017-2021

Months	Average relative humidity (%)	Average temperature ($^\circ\text{C}$)	Rainfall (mm)
January	73.14	-8.98	11.52
February	69.38	-5.76	10.16
March	65.02	0.84	34.00
April	56.28	6.24	35.76
May	56.06	11.92	76.48
June	53.80	16.18	60.20
July	53.28	19.24	58.64
August	48.88	19.00	36.52
September	49.54	15.12	29.48
October	63.06	8.30	43.60
November	70.12	1.16	26.04

SD: Standard deviation

Table 2. Seasonal variation of vital signs of dogs in Kars Province

Parameter	Winter (Mean \pm SD)	Summer (Mean \pm SD)	P value
Body Temperature ($^\circ\text{C}$)	38.21 ± 0.59	38.8 ± 0.71	0.014
Respiratory (breath/min)	29.83 ± 6.89	44.17 ± 19.75	0.025
Pulse (beats/min)	92.67 ± 11.16	111.00 ± 23.22	0.016

SD: Standard deviation

Table 3. Seasonal changes of hematological values of mixed breed dogs

Parameter	Winter (Mean \pm SD)	Summer (Mean \pm SD)	p-value
WBC ($10^9/\text{L}$)	17.88 ± 6.32	14.34 ± 4.15	0.034
MON ($10^9/\text{L}$)	0.50 ± 0.17	0.45 ± 0.11	0.290
GRA ($10^9/\text{L}$)	12.13 ± 6.08	10.13 ± 3.32	0.480
RBC ($10^{12}/\text{L}$)	7.30 ± 0.82	7.38 ± 0.71	0.806
HCT (%)	53.38 ± 5.88	57.20 ± 5.84	0.123
MCH (pg)	24.93 ± 1.16	21.80 ± 2.12	0.005
MCHC (g/dL)	34.13 ± 1.27	28.13 ± 2.22	0.002
HB (g/dL)	18.24 ± 2.04	16.05 ± 1.44	0.006
THR ($10^9/\text{L}$)	199.42 ± 107.33	206.83 ± 99.60	0.583

SD: Standard deviation, WBC: White blood cell, MON: Monocyte, GRA: Granulocyte, RBC: Red blood cell, HCT: Hematocrit, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, HB: Hemoglobin, THR: Thrombocyte

Table 4. Seasonal changes of biochemical values of mixed breed dogs

Parameter	Winter (Mean ± SD)	Summer (Mean ± SD)	p-value
ALT (U/L)	70.72 ± 33.85	87.69 ± 11.40	0.117
AST (U/L)	28.10 ± 14.73	35.33 ± 9.96	0.117
ALP (U/L)	41.99 ± 24.02	71.13 ± 49.80	0.060
CK (U/L)	212.91 ± 81.19	312.81 ± 95.02	0.050
Urea (mg/dL)	31.38 ± 7.28	37.09 ± 5.77	0.027
TP (g/dL)	6.31 ± 0.47	6.77 ± 0.51	0.030
Albumin (g/dL)	2.98 ± 0.13	3.17 ± 0.19	0.022
Globulin (g/dL)	3.33 ± 0.49	3.60 ± 0.54	0.121
Cholesterol (mg/dL)	173.35 ± 13.95	157.69 ± 14.52	0.021
TG (mg/dL)	63.20 ± 7.81	56.33 ± 7.77	0.055
Glucose (mg/dL)	125.11 ± 20.80	79.42 ± 10.55	< 0.001
Creatinine (mg/dL)	0.67 ± 0.12	0.88 ± 0.15	< 0.001

SD: Standard deviation, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline phosphatase, CK: Creatine kinase, TP: Total protein, TG: Triglyceride

DISCUSSION

The findings reveal the differences in dogs between the winter and summer. Examining the effect of the seasons on dogs, indicated that body temperature, respiratory rate, and heart rates are significantly lower during the winter. The findings reveal that dogs reduce their metabolic activities in cold weather and activate adaptation mechanisms to ensure thermal regulation. Similarly, the changes in hematological and biochemical parameters are also noteworthy.

Evaluation of hematological, biochemical, and vital parameters provides useful information in the diagnosis of various diseases. Additionally, these parameters are important for the evaluation of some metabolic and physiological processes (Azeez *et al.*, 2022). Heatstroke (Drobatz, 2015) and hypothermia (Todd, 2015) have numerous physiological effects, and low environmental temperatures can lead to various physiological changes (Ji *et al.*, 2021).

The high temperature and relative humidity of the environment have been stated as the primary cause of heat stress (Azeez *et al.*, 2022). In a study conducted on dogs, it was determined that different environmental temperatures did not affect vital parameters up to a certain level, but at very high temperatures (45°C), significant increases in body temperature, pulse, and respiratory rate were observed (Al-Shammari *et al.*, 2019). In the present study, it was determined that vital values were significantly lower in the assessments conducted during the winter compared to those conducted in the summer. The primary factor contributing to this change is believed to be influenced by ambient temperature through a mechanism mediated by receptors in the hypothalamus and skin.

Azeez *et al.* (2022) noted that the total leukocyte count in dogs is lower in environments with high ambient temperature and high relative humidity compared to environments with high ambient temperature and low relative humidity. Studies conducted on sheep (Khalil *et al.*, 2022) and horses (Fernando de Souza *et al.*, 2018) have reported that the total leukocyte count in winter is higher compared to summer. Mohammed *et al.* (2017) conducted a study on dogs and reported that the total leukocyte count in the winter is statistically insignificant compared to the summer. Accordingly, the total leukocyte count was determined to be significantly higher in winter compared to summer. The larger size of lymphatic organs during the winter season (Nelson and Demas, 1996), or the stress conditions resulting from lower ambient temperatures during the winter months, have been cited as the reasons for this phenomenon.

The temperature changes occurring throughout the year have been reported as a physiological stress factor. The biological systems of animals are affected by this condition (Khalil *et al.*, 2022). Season, ambient temperature, rainfall amount, and humidity changes are important factors affecting hematological parameters (Mohammed *et al.*, 2017). It has been reported that heat stress may cause precipitation in Hb in erythrocytes and therefore a decrease in Hb concentration (Karthik *et al.*, 2021). Additionally, the absence of Hb's essential components in the diet and the body's ability to absorb these components directly influence Hb levels (Šimák-Líbalová *et al.*, 2013). Khalil *et al.* (2022) conducted a study on sheep that indicated the Hb levels were found to be lower in the winter compared to the summer. However, Hb levels were shown to be greater in the winter in donkeys (Longodor *et al.*, 2020). Accordingly, winter Hb levels were higher than summertime levels. It has been proposed that the potential cause for this phenomenon could be the physiological strain induced by temperature changes (extreme cold or heat) experienced during different seasons and daily temperature fluctuations. This strain may result in insufficient absorption of the essential elements required for Hb at the intended

level. In a study conducted on sheep by Khalil et al. (2022) and dogs (Mohammed et al., 2017), MCH and MCHC values were found to be high in the winter, while in horses (Fernando de Souza et al., 2018) they were found to be low in the winter. In the present study, higher MCH and MCHC values were recorded during the winter analysis compared to the summer. The decrease observed during the summer season was associated with a decrease in Hb levels.

It is crucial to analyze the biochemical parameters in serum and plasma samples collected post-blood sampling for the clinical and metabolic assessment of animals (Çınar et al., 2010).

Mohammed et al. (2017) have reported that the serum urea level in dogs is higher in summer compared to winter, while Longodor et al. (2020) noted that it is higher in donkeys in the winter. In the present study, high serum urea levels were recorded in the summer season. It has been suggested that this change is attributed to fluid loss, which is shaped by the increasing ambient temperature during the summer season.

Creatinine moves into the blood as the end product of the nonenzymatic hydrolysis of creatine phosphate in the muscles, is distributed homogeneously into body fluids, and is filtered freely through the glomerulus (Turgut, 2000). Donkeys' serum creatinine levels were shown to be greater in the winter than in the summer, according to Longodor et al. (2020). Sheep's summertime creatinine levels were shown to be high in another investigation, and this was associated with decreased blood flow to the kidneys due to heat stress (Rathwa et al., 2017). In the current study, serum creatinine level was found to be higher in summer than in winter. It was determined that the values obtained during the winter and summer fall within the reference ranges (Turgut, 2000; Erkilic, 2023). The transition between seasons is linked to dehydration caused by rising temperatures during the summer months. Based on biochemical parameters, the lower levels of blood urea, total protein, albumin, and creatinine during the winter are likely associated with adaptation mechanisms in metabolic activities occurring in response to cold stress.

Albumin, which constitutes approximately 50% of plasma proteins, contributes to plasma oncotic pressure. Changes in oncotic pressure are thought to control its hepatic synthesis (Turgut, 2000). Increased plasma albumin concentration due to dehydration decreases as a result of decreased hepatic synthesis, increased breakdown, or excessive loss through urine or intestines (Turgut, 2000). It has been reported that the serum total protein level in dogs decreases in cases of severe liver damage and long-term protein malnutrition, and its levels increase in cases of shock and dehydration (Kalaycioğlu et al., 1995). According to Mohammed et al. (2017), the stress of the hot weather caused an increase in total protein levels in the summer compared to the winter in dogs, while albumin levels were recorded to be at similar levels. In the presented study, serum total protein and albumin levels were found to be higher during the summer compared to the winter. The seasonal variation of these parameters, which fall within the reference ranges (Turgut, 2000; Erkilic, 2023), was associated with fluid loss occurring during the summer season.

There are studies in which glucose levels are determined in different animal species at different ambient temperatures, humidity levels, and seasons (Azeez et al., 2002; El-Shahat Attia, 2016; Longodor et al., 2020). Glucose analyses during the winter have noted that glucose levels are higher compared to the summer in dairy cows (Yıldız and Kızıl, 2011), sheep (Rathwa et al., 2017), and donkeys (Longodor et al., 2020). In the current study, glucose levels in dogs were also higher during the winter. Accordingly, ambient temperature may adversely affect blood glucose levels, however, the increase in temperature during the summer negatively affects food consumption in dogs. Intensive feeding by animal owners during the winter to mitigate the effects of cold conditions is effective in influencing changes in glucose levels.

Previous studies on different animal species (donkey, sheep, bull, dog) indicated that cholesterol levels differ in summer and winter, and cholesterol levels were found higher in winter than in summer (Farooq et al., 2017; Mohammed et al., 2017; Rathwa et al., 2017; Longodor et al., 2020). In the presented study, the cholesterol level in dogs was recorded to be higher in winter compared to the summer. The cholesterol levels, falling within the reference range (Turgut, 2000; Erkilic, 2023), reveal an elevation during the winter season. This phenomenon has been linked to heightened feeding activities in winter months, a partial decrease in food intake during summer months due to rising ambient temperature, or as an adaptation to seasonal conditions. Among studies that investigated the physiological effects of thermal changes, such as heat stress, summer-winter conditions, and seasonal differences in various animals, including dog, sheep, and horse species, the lowest atmospheric temperature reported was not below zero degrees, although the study regions reflected winter conditions (Mohammed et al., 2017; Rathwa et al., 2017; Fernando de Souza et al., 2018; Al-Shammari et al., 2019). The most notable feature of the present study is that the temperature during the winter was cold adequate (-8.4°C) to affect the physiological resilience of the animals.

CONCLUSION

The winter has a significant impact on the body temperature, respiratory rate, heart rate, some hematological (WBC, MCH, MCHC, HB), and biochemical parameters (urea, TP, albumin, cholesterol, glucose, creatinine) of dogs. The

obtained results may help to understand the physiological (heart rate, respiratory rate, and body temperature), hematological, and biochemical changes that dogs are exposed to during intense winter conditions and may contribute to the adaptation of veterinary health care to these conditions. It was concluded that hematological and biochemical parameters may vary under the influence of seasonal conditions and these factors should be considered in clinical examinations. The present study emphasizes the importance of seasonal variations in assessing the health status of dogs. Future studies focusing on genetics, breed, nutrition, and environmental factors will contribute to this issue in more detail.

DECLARATIONS

Funding

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Ethical considerations

All authors have checked and confirmed the ethical concerns regarding the originality of collected data, and written sentences of this article before submission to the journal.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

Consent to publish

All authors agree to the publication of this manuscript.

Authors' contributions

Ekin Emre Erkiliç took part in the design, idea, collection of samples, and writing of the article. İsa Özyaydın took part in the design, idea, and writing of the article. Oğuz Merhan took part in sample analysis. Mert Sezer, Yusuf Umut Batı, Celal Şahin Ermutlu, and Ali Haydar Kırmızıgül took part in the sample collection. All authors read and approved the final version of the manuscript.

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