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# The Influence of Thermal Stress on Serum Biochemical Profile in Sheep

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## ABSTRACT

**Background:** Climatic factors, such as high temperature, high relative humidity, can induce a thermal stress in animals. The phenomenon of mammalian sensitivity to thermal stress, especially in small ruminants, is widely reported in the literature. The present study aimed to analyze temperature and humidity effects on serum metabolic profile and cortisol concentration in sheep.

**Methods:** The experiment was conducted on 40 adult, non-lactating and non-pregnant Suffolk sheep from December 2018 to December 2019. The subgroups were formed by age (two groups): twenty sheep were about 1.5 years old (Group 1) and other twenty - about 3 years old (Group 2). Based on the value of the temperature-humidity index, the following three subgroups were formed: 1) temperature humidity index ≥20; 2) 20> temperature-humidity index >10 and 3) temperature-humidity index <10. Blood cortisol concentration and biochemical parameters were measured once per month on the same day, in identical animals.

**Result:** The analysis of biochemical parameters revealed that Group 2 showed significantly higher values for creatinine, phosphorus, zinc and cortisol. All blood indicators, except iron, phosphorus and total bilirubin, were dependent on THI concentration. The highest positive correlation coefficient of cortisol was calculated with urea and total protein. It is concluded that both cortisol and biochemical parameters play a significant role in thermal stress reactions in the Suffolk sheep.

**Key words:** Biochemical parameters, Cortisol, Sheep, Temperature-humidity index, Thermal stress.

## INTRODUCTION

The severity of thermal stress should not be determined only by temperature factor: it is also important to include the humidity value as it has a significant impact on perceptible temperature. To include both factors, the use of the temperature-humidity index (THI) has been mentioned by numerous authors (Behera *et al.* 2018). Sheep have developed a set of mechanisms that protect them from overheating. When the physiological mechanisms fail to alleviate the effect of heat load, the body temperature may increase to a point at which animal well-being can be compromised. The change of environmental temperature has a significant impact on physiological processes (Behera *et al.* 2018). Thermal stress causes the decrease in feed intake, disturbance in the metabolism of water, protein, energy and mineral balance, hormonal secretions and blood metabolites (Shahar *et al.* 2017). Cortisol, secreted by the adrenal glands, stimulates physiological changes in the body leading to increased tolerance of the animals to high temperature-induced stress (Priyanka *et al.* 2013). It was found that the value of cortisol significantly increased in animals exposed to high temperature and gradually decreased during long-term exposure. It was observed that animals often differ in their tolerance and susceptibility to thermal stress. The animals adapted to hot/cold climatic conditions should show least variation in their physio-biochemical traits when raised under such conditions (Silanikove, 2000).

Biochemical analysis provides reliable information on the health condition of sheep. It is an important variable in the assessment of adaptive and productive capacity of breeds under unfavourable environmental conditions (Bezerra *et al.* 2017). Apparently, in sheep hyperthermia conditions during prolonged periods reduce blood metabolite levels related to energy metabolism and increase metabolite concentrations linked to protein metabolism (Bezerra *et al.* 2017). Meanwhile, under short-term thermal stress, the metabolites associated with energy metabolism may increase to ensure high availability of energy substrates required at the time of physiological adjustments (Shahar *et al.* 2017). The aim of the study was to track the influence of temperature and humidity effects on serum metabolic profile.

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## MATERIALS AND METHODS

### Location of study

The experiment was carried out in the eastern region of Europe, Lithuania, from December 2018 to December 2019. The sheep farm is located at 54.776651 latitude and 24.820980 longitude. The climate of the country is transitional between the maritime typical of Western Europe and the continental type found farther east, characterized by warm, dry summers and fairly severe winters**.** In terms of climate change, the climate is warming every year, summers are hotter and winters are less severe. The average ambient temperature during experimental year was 10.50 ±1°C, the relative humidity was 77.60 ±2%. The average values of daily humidity and air temperature in the farm area were obtained from the adjacent weather station (2 km away). Measurements were taken every hour. The daily THI values were calculated according to the equation (a) by Marai *et al.* (2007):

$$THI = T-(0.31- 0.0031\times RH) \times (T-14.4) \quad (a),$$

where,

T is the dry-bulb temperature and RH is the relative humidity. The average daily values of temperature and relative humidity were used to calculate the daily values of THI that were analysed in this study.

### Experimental design, feeding and housing

The experiment was conducted on the Suffolk sheep. Fourty sheep were selected based on the following criteria: not pregnant during the entire study period, clinically healthy. These parameters were determined by a precise clinical examination (an average rectal temperature 38.5±0.2°C, rumen contractions three to four times per two minutes; no signs of a clinical disease). According to the test data, the groups were formed by age (two groups) and THI (three subgroups). Twenty of these sheep were 1.5±0.3 years old with similar body weight (35±2 Kg; Group 1) and other twenty sheep were 3.5±0.3 years old with similar body weight (45±3 Kg; Group 2). According to the THI value, the following three subgroups were formed: 1) THI≥20; 2) 20>THI>10 and 3) THI<10. All sheep were kept under same conditions of the loose housing system and throughout the year, all sheep were fed at the same time the same balanced ration according to their physiological needs. The grazing season was from April to October. The pastures were predominantly grasses and legumes. In addition, in summer, all sheep on the farms at all times had *ad libitum* access to the complete mineral mixture blocks. In winter, the ewes were kept in barns and fed the grain mixture (300 g/day), grass hay (Timothy grass, red clover, white clover, alfalfa, ryegrass, quack grass). Chemical composition of forage was as follows: 94% dry matter (DM), 13% crude protein (CP), 9% ash, ether extract 1.2%, neutral detergent fiber 37.2% and acid detergent fiber 21.4%. Water was provided *ad libitum*.

### Measurements

Blood samples were taken between 7:00-8:00 a.m. after overnight fasting by jugular venipuncture, using vacuum tubes without any anticoagulant. Blood samples were taken once per month on the same day from the identical animals. In the study, 520 samples were tested (13 months x 40 animals). Further research was performed in the Lithuanian University of Health Science, Veterinary Academy. The samples were refrigerated at 8°C and transported immediately to the laboratory for analysis. Within an hour, the blood samples were centrifuged for 10 min. at 3500 rpm and blood serum was collected and stored -20°C. The blood biochemical parameters - calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), urea, total proteins (TP), glucose (Gl), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total bilirubin (TBIL), albumin (Alb), creatinine (Crea) and lactate dehydrogenase (LDH) were measured using commercial kits on the Selectra Junior analyser (AC Dieren, The Netherlands, 2006). Cortisol level was measured by an automated analyzer TOSOH® AIA-360 (South San Francisco, CA), which uses a competitive fluorescent enzyme immunoassay. Accuracy and performance data for human and canine T4 and cortisol, including analyte recovery and dilutional studies, had been previously evaluated. Daily checks, calibration curves and maintenance procedures were performed as described in the System Operator's Manual.

### Statistical analyses

Statistical analysis of the biochemical blood indicators was carried out using the SPSS 25.0 software (SPSS Inc, Chicago, IL, USA). Normal distributions for all blood indicators were assessed by the Kolmogorov-Smirnov test. The results of statistical analysis were recorded as the mean±standard error. The linear relationship between biochemical blood indicators were evaluated using Pearson's correlation. The t-test can be used to determine if the means of two sets significantly differ. A probability of less than 0.05 was considered as being significant ($P < 0.05$).

The research was performed according to the Law of the Republic of Lithuania No. 8-500 on Protection, Keeping and use of Animals, dated 06/11/1997 (Valstybės žinios (Official Gazette) No. 108 dated 28/11/1997) and orders of the State Veterinary Service of the Republic of Lithuania on Breeding, Care and Transportation of Laboratory Animals (No. 4-361, dated 31/12/1998) and use of Laboratory Animals for Scientific Tests (No. 4-16, dated 18/01/1999). The study approval number was PK014606.

## RESULTS AND DISCUSSION

### Relation of the blood biochemical parameters with the temperature-humidity index (THI)

The interpretation of biochemical profiles is complex due the mechanisms that control the blood level of various metabolites (Stevanović *et al.* 2015). Table 1 represents that with an increase in the THI value (from Subgroup 1 to Subgroup 3) in Group 1, the values of blood urea (1.71-2.4 times; $P<0.05$), P (1.16-1.24 times; $P<0.05$), GGT (1.04-

**Table 1:** Evaluation of the effect of THI on blood biochemical parameters in sheep groups.

| Indicator | THI 1 | | THI 2 | | THI 3 | |
|---|---|---|---|---|---|---|
| | M | SE | M | SE | M | SE |
| **Group 1** | | | | | | |
| Urea (mmol/l) | 4.80[a] | 0.26 | 8.23[b] | 0.30 | 11.50[c] | 0.18 |
| Gl (mmol/l) | 3.19[a] | 0.06 | 2.48[b] | 0.08 | 2.02[c] | 0.14 |
| AST (µkat/l) | 125.02[a] | 3.20 | 107.49[b] | 2.01 | 120.54[a] | 6.63 |
| Fe (µg/dl) | 139.21[a] | 5.24 | 136.48[a] | 3.93 | 139.48[a] | 8.20 |
| Crea (µmol/l) | 128.92[a] | 3.17 | 130.10[a] | 7.18 | 107.05[a] | 2.38 |
| Ca (mmol/l) | 2.51[a] | 0.04 | 2.70[b] | 0.02 | 2.71[b] | 0.02 |
| Mg (mmol/l) | 0.79[a] | 0.01 | 0.79[a] | 0.01 | 1.07[b] | 0.26 |
| P (mmol/l) | 1.52[a] | 0.04 | 1.77[b] | 0.03 | 1.89[b] | 0.08 |
| TP (g/l) | 64.87[a] | 1.06 | 71.04[b] | 0.84 | 70.11[b] | 0.80 |
| TBIL (µmol/l) | 2.33[a] | 0.10 | 2.89[a] | 0.46 | 2.45[a] | 0.08 |
| Alb (g/l) | 27.37[a] | 0.31 | 29.00[b] | 0.29 | 28.77[b] | 0.41 |
| Cu (µg/dl) | 137.02[a] | 3.94 | 121.86[b] | 3.27 | 101.75[c] | 4.25 |
| Zn (µg/dl) | 193.20[a] | 2.34 | 181.24[b] | 2.59 | 194.63[a] | 2.63 |
| GGT(µkat/l) | 52.28[a] | 1.98 | 54.51[a] | 1.12 | 60.04[b] | 2.14 |
| LDH (µkat/l) | 1119.57[a] | 29.86 | 1018.72[b] | 20.68 | 1155.92[a] | 58.09 |
| Cortisol (µg/dl) | 1.10[a] | 0.10 | 1.90[b] | 0.13 | 2.34[c] | 0.14 |
| **Group 2** | | | | | | |
| Urea (mmol/l) | 4.08[a] | 0.16 | 8.96 [b] | 0.33 | 12.24[c] | 0.29 |
| Gl (mmol/l) | 3.41[a] | 0.11 | 2.30 [b] | 0.08 | 2.26[b] | 0.17 |
| AST (µkat/l) | 120.07[a] | 3.67 | 106.01 [b] | 1.90 | 108.32[b] | 3.17 |
| Fe (µg/dl) | 142.61[a] | 7.98 | 127.12 [a] | 3.44 | 129.40[a] | 7.42 |
| Crea (µmol/l) | 135.12[a] | 4.08 | 113.64[b] | 2.19 | 103.00[c] | 1.90 |
| Ca (mmol/l) | 2.53[a] | 0.04 | 2.67[a] | 0.04 | 2.66[a] | 0.03 |
| Mg (mmol/l) | 0.81[a] | 0.01 | 0.80[a] | 0.01 | 0.82[a] | 0.01 |
| P (mmol/l) | 1.61[a] | 0.16 | 1.53[a] | 0.03 | 1.44[a] | 0.05 |
| TP (g/l) | 68.53[a] | 1.42 | 71.03[a] | 1.16 | 73.63[b] | 0.79 |
| TBIL (µmol/l) | 2.33[a] | 0.09 | 2.48[a] | 0.06 | 2.48[a] | 0.09 |
| Alb (g/l) | 27.81[a] | 0.26 | 28.32[a] | 0.35 | 28.73[a] | 0.44 |
| Cu (µg/dl) | 153.17[a] | 6.82 | 119.05[b] | 3.07 | 103.35[c] | 2.95 |
| Zn (µg/dl) | 194.71[a] | 1.83 | 168.37[b] | 2.62 | 189.12[a] | 3.95 |
| GGT (µkat/l) | 48.88[a] | 2.25 | 57.85[a] | 1.44 | 59.95[c] | 2.39 |
| LDH (µkat/l) | 1073.21[a] | 38.82 | 1058.75[a] | 27.70 | 1062.25[a] | 60.62 |
| Cortisol (µg/dl) | 1.84[a] | 0.22 | 2.57[b] | 0.18 | 3.08[c] | 0.42 |

[abc]Rows means with different superscripts differ significantly at $P<0.05$.

1.15 times; $P<0.05$ ) and cortisol (1.73-2.13 times; $P<0.05$) were increased. Increased activity of GGT in clinically healthy sheep can be considered as a consequence of an intensification of metabolic processes and a response of the body to the negative energy balance (Stevanović *et al.* 2015). In addition, increased activity of this enzyme in clinically healthy sheep may be caused by higher humidity and higher temperature, related to the increased degradation of glutathione (Hodžić *et al.* 2011). Likewise, it was observed that in Group 2, the values of blood urea (2.20-3.00 times; $P<0.05$), TP (1.04-1.07 times; $P<0.05$), GGT (1.18-1.23 times; $P<0.05$) and cortisol (1.40-1.67 times; $P<0.05$) increased and the concentration of Gl (1.48-1.51 times; $P<0.05$), Crea (1.19-1.31 times; $P<0.05$) and Cu (1.29-1.48 times; $P<0.05$) decreased in the blood. Some studies have shown that in a high-temperature environment, the values of glucose and cholesterol in the blood decrease, which is an indicator of failure in homeostasis (Ribeiro *et al.* 2018). The maintenance of stable concentration of glucose in the blood is regulated by the liver, extrahepatic tissues and hormones namely insulin, glucagon, adrenaline, cortisol and thyroid hormones (Ribeiro *et al.* 2018). The serum glucose concentrations of the Suffolk sheep was higher in cold conditions and decreased in higher temperature 1.29-1.58 times ($P<0.05$) in Group 1 and 1.48-1.51 times ($P<0.05$) in Group 2. The present results were similar to the findings of of Soveri *et al.* (1992) in reindeer calves and Nazifi *et al.* (1999) in dromedary camels. Glucose concentrations may alter with the secretion of catecholamines and may also increase secondarily as a result of the stress of muscle and

**Table 2:** Correlation of blood cortisol with biochemical parameters by sheep groups and THI level.

| Indicator | Group 1 | Group 2 | THI 1 | THI 2 | THI 3 |
|---|---|---|---|---|---|
| Urea (mmol/l) | 0.291** | 0.216** | -0.132 | 0.194** | 0.015 |
| Gl (mmol/l) | -0.158* | -0.250** | 0.500** | -0.164* | -0.223 |
| AST (µkat/l) | 0.069 | -0.0187* | 0.039 | 0.049 | -0.217 |
| Fe (µg/dl) | 0.077 | 0.326** | 0.209 | 0.224** | 0.104 |
| Crea (µmol/l) | 0.033 | 0.188* | 0.235* | 0.094 | -0.153 |
| Ca (mmol/l) | 0.161* | 0.017 | 0.309** | -0.032 | -0.032 |
| Mg (mmol/l) | 0.101 | 0.098 | 0.045 | 0.068 | 0.063 |
| P (mmol/l) | .0.225** | 0.096 | 0.148 | 0.071 | 0.118 |
| TP (g/l) | 0.293** | 0.250** | 0.432** | 0.268** | -0.056 |
| TBIL (µmol/l) | -0.009 | 0.037 | 0.020 | -0.019 | -0.021 |
| Alb (g/l) | 0.190** | 0.107 | 0.240* | 0.156* | -0.153 |
| Cu (µg/dl) | 0.031 | 0.072 | 0.107 | 0.244** | -0.132 |
| Zn (µg/dl) | 0.137* | 0.230** | 0.124 | 0.257** | -0.044 |
| GGT (µkat/l) | 0.040 | 0.026 | -0.048 | -0.071 | 0.129 |
| LDH (µkat/l) | 0.044 | 0.032 | 0.139 | 0.112 | -0.171* |

Corrrelation coefficient significant at *$P$<0.05 and **$P$<0.01.

liver enzymes induced by myopathy and hypoxia (Durak *et al.* 2015). We found significantly ($P$<0.05) increased values of urea. Such increase in value may be caused due to reduced blood flow toward kidney in thermal stress conditions. Similar findings of increased urea during summer season have been reported by Ghosh *et al.* (2013). In the present study, the average concentration of total protein was by 1.04-1.07 times ($P$<0.05) higher in Group 2, compared to Group 1. The impact of age and THI on total serum protein was also observed in some sheep breeds, such as Merino Landschaf (Antunović *et al.* 2004) and Chios (Roubies *et al.* 2006).

**Relation of blood biochemical parameters with THI and cortisol concentration**

The concentration of cortisol in the blood increased significantly in animals exposed to high temperature and high humidity, indicating thermal stress. However, blood cortisol concentrations vary due to different factors, including ambient temperature and/or humidity, management and physiological conditions (Priyanka *et al.* 2013). The relationship between temperature and humidity and increased cortisol secretion is sparsely documented in ruminants. High temperatures cause elevation of blood cortisol concentration, decreasing the rate of metabolic heat production (Sejian *et al.* 2010). Analysis of correlations between blood cortisol and estimated blood biochemical parameters (Table 2) shows the same direction of change in all parameters except AST. The statistically reliable results obtained in the study comfirmed that cortisol concentration increases are directly related to the corresponding changes in ambient temperature and humidity. It was found that with an increase in the THI level (from Subgroup 1 to Subgroup 3) in Group 1, values of cortisol increased by 1.73-2.13 times ($P$<0.05) and in Group 2, the cortisol level increased by 1.40-1.67 times ($P$<0.05). Nevertheless, the study performed on the Indian sheep (Ashutosh *et al.* 2001) suggested that higher cortisol values may be correlated not only with high ambient temperature. The highest positive correlation coefficient of cortisol was calculated with urea (r = 0.22-0.29, $P$ <0.001) and TP (r = 0.25-0.29, $P$ <0.001), a negative relationship between blood Gl and cortisol was found in both groups (r = - 0.16-0.25, $P$ <0.05-0.001) (Table 2). In animals of Subgroup THI 1, cortisol positively correlated with Gl, Ca, TP, Zn ($P$ <0.01), Crea and Alb ($P$ <0.05). Cortisol in the Subgroup THI 2 was positively associated with urea, Fe, TP, Cu, Zn ($P$ <0.01) and Alb ($P$ <0.05). THI class 3 revealed a negative relationship between cortisol and Gl, Fe, Crea, Ca, TP and TBIL, Alb, Cu, Zn and LDH, but these correlations were statistically unreliable. The association of cortisol with blood indicators was dependent on THI values in Subgroup THI 1 and Subgroup THI 2, but correlations in Subgroup THI 3 were statistically unreliable. The THI index had no effect on blood parameters in Subgroup THI 3. In domestic animals, including sheep, the highest values of cortisol were measured in the morning (Hrković-Porobija *et al.* 2017). In the study, all samples were taken early in the morning, between 7:00-8:00 a.m. Cortisol secretion stimulates physiological modifications that enable an animal to tolerate the stress caused by a hot environment and the initial reactions of the animal to thermal stress are emotional rather than responding thermoregulation (Al-Samawi *et al.* 2014). Finally, the stress reactions were summarized by Olsen *et al.* (2006), who argue that despite hundreds of thematic publications, the relevant physiological mechanisms until today remain unclear and thus further heat studies should be carried out.

## CONCLUSION

According to the aim of the current study - to track the impact of temperature and humidity effects on serum metabolic profile and also to check their correlation with cortisol concentrations, it can concluded that the higher THI is associated with significantly increased activity of GGT and

concentration of urea, P, TP and cortisol as well as with decreased value of Gl, Cu and Crea. The analysis of correlations between blood cortisol and estimated blood biochemical parameters shows the same direction of change in all parameters except the activity of AST.

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