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Effect of thermal stress on physiological and blood parameters in merino sheep

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Abstract

Fifteen sheep where placed in climatic chamber and exposed to a high temperature (30°C). Then, the air movement was induced in order to examine its soothing effect on heat stress. The physiological reactions like respiratory and heart rates, as well as the morphologic, biochemical parameters and cortisol levels in blood were examined. It was found that under heat stress conditions, the respiratory rate increased up to 96.43 breaths/min, heart rate up to 107.79 beats/min, and white blood cells count decreased to 9.12 k/μL. The increased level of potassium, chlorine, and calcium was also observed. The increased air movement resulted in thermal stress soothing. A decrease in respiratory rate, heart rate, and cortisol concentration was observed. The study demonstrated that heat stress leads to serious changes in physiological and blood parameters in sheep but this effect can be minimised by air movement.

Key words: sheep, heat stress, air movement, physiological parameters, cortisol.

Introduction

Sheep farming still remains a very important branch of agriculture, especially in developing countries, as sheep have good adaptation ability and are resistant to hard environmental conditions. Thermal stress leads to drastic changes in the body functions. It implies a reduction in productivity: decrease in reproductive functions, milk yield, and weight gain (5).

According to Taylor (17), sheep thermo-neutral zone is set between -12°C and 32ºC (full fleece sheep). Range of the thermo-neutral zone depends on many factors like breed of the animal, its age, body weight, feeding, health status, and physiological state (7). To determine the severity of heat stressor, it is important to use not only temperature measurements, but also humidity level, as it has a significant impact on the perceptible temperature. For this purpose, the study on the temperature-humidity index (THI) was determined.

THI allows the integration of temperature and humidity into one value, which allows an objective comparison of environmental conditions. Currently, the index is broadly used, allowing the investigators to understand better the effects of thermal conditions. However, the index should not be treated as the absolute value to determine thermal nuisance. It is important to be aware of a significance of factors, such as air movement or sun radiation on animals' physiological status.

High environmental temperature forces animals to induce several thermoregulatory mechanisms. In sheep, fleece acts as a protective barrier and hinders evaporation of water from the body, thus reducing heat loss trough sweating. Therefore, evaporation while breathing becomes much more important. Heat stress causes many physiological changes in sheep, which includes a decrease in feed intake, disturbance in the metabolism of water, protein, energy, and mineral balance, hormonal secretions, and blood metabolites (5, 9). Environmental temperature change has a significant impact on the physiological processes of the body. The rate of oxygen consumption increases in direct proportion to the temperature. It is assumed that the increase of the temperature of 10°C causes a two or even a threefold increase in oxygen consumption speed (13). To fulfil the increased oxygen demand of the organism, animals are forced to increase gas exchange in the lungs by increased respiratory rate. Breathing, however, is used not only to remove the CO_2 from the body and supply O_2, but also to prevent overheating of the animal. Sheep under thermally neutral conditions (12ºC) lose about 20% of the heat by water vapour contained in the exhaled air. This value may increase up to about 60% at a high temperature (35°C). Respiratory rate (RR) can be a good indicator of heat stress (5). The physiological RR in sheep is 25-30 breaths per minute. An increase in frequency above 40 breaths per minute can be considered as panting and it is used to increase the heat loss by exhaling water vapour in breath. In severe thermal stress, the RR reaches 300 breaths per minute. Breathing enables to remove the excess heat in sheep, resulting in heat loss (14). Thermal stress determination based on respiratory rate has advantages due to the ease of assessment. Silanikove (14) describes the guidelines presented in Table 1. It is not necessary to use any equipment except the timer, and observation can be made even from a distance.

Table 1. Thermal stress level based on the respiratory rate (14)

Breaths/minute	Level of heat stress
40-60	Low
60-80	Medium
80-120	High
>200	Severe heat stress

Heart rate is another parameter, which increases in animals exposed to high temperatures, leading to increased blood flow from the interior of the body into the subcutaneous layer. This provides a chance of losing a greater amount of heat by conduction, convection, and radiation (1). The amount of blood supplied to the heart, respiratory muscles, and the earlobes is increasing, but in a smaller extent, the increase is observed in internal organs such as the intestines (12). Body temperature measured per rectum is often used as a practical and representative method for measuring internal body temperature (13). Sheep body temperature stays within the range of 38.3°C to 39.9°C under thermoneutral conditions. It is recognised that the body temperature of 42ºC and higher is dangerous for the sheep (14). It is also assumed that an increase in the parameter over those standards is a sign that the animal is not able to cope with the high ambient temperature (18).

Cortisol, secreted by the adrenal glands, stimulates physiological changes in the body, which allow the animal a better tolerance to the stress caused by high temperature (2). It has been found that level of cortisol significantly increases in animals exposed to high temperature and gradually decreased during long-term exposure. It is assumed that a gradual decrease in cortisol levels during chronic heat stress is the result of animal's adaptation to the acute thermal conditions (14).

The aim of this study was to examine physiological reactions occurring in animals during heat stress and to determine whether slight air movement can lead to heat stress mitigation. That could have practical implications in sheep farming practices and farm building architecture.

Material and Methods

Fifteen clinically healthy Merino rams, 12 months of average age and 45 kg of average weight, were used. The animals were selected for the experiment according to similar weight, constitution, and fleece length.

Sheep were placed in an experimental room equipped with air-conditioning and heating units. To monitor microclimatic conditions, the thermo-hygrometers, as well as a device measuring the concentration of ammonia, carbon dioxide, and hydrogen sulfide were placed at a central point above the animals. Cameras connected to the computer system enabled the observation and recording of animals' activity and behaviour. System was set to 14 h of day light. The animals had a constant access to water and forage (hay). Feeding was made with nutritive fodder in the form of oats: 0.2 kg/individual/day. During the experiment, detailed measurements of physiological parameters of the animals were made. Heart rate (HR) and respiratory rate (RR) were monitored with a stethoscope. Body temperature was measured by an electronic thermometer (per rectum), blood samples for morphological and biochemical tests for evaluation of cortisol levels were collected every day. Morphological parameters were evaluated with Pentra 400, Horiba ABX unit and biochemical parameters were evaluated with ABC Vet, Horiba ABX unit. Parameters such as air temperature, relative humidity, and air movement were constantly measured and monitored with the use of software "SCADA PRO" designed for environmental data collection. The THI index was calculated according to the Hahn formula (6):

$$THI = 0.81\ db\ ºC + RH\ (db\ ºC - 14.4) + 46.4;$$

where: db ºC – dry bulb temperature, RH – relative humidity (RH%)/100.

The results of the experiments were statistically analysed using a Duncan test. Statistically significant differences ($P \leq 0.05$) and highly significant differences ($P \leq 0.01$) are indicated with a superscript in the tables presenting the results obtained.

The first three weeks were designed for acclimatisation of the animals. During this time the blood samples were collected and basic measurements were made to determine whether the animals selected for the experiment were fully healthy and in a physiological equilibrium state. Then, the experiment was divided into three stages (environmental parameters for each stage are summarized in Table 2).

The first experimental phase lasted 14 d. Sheep were provided with a sufficient level of welfare and neutral conditions including temperature and humidity. During the day, the average temperature was 20.95°C, average relative humidity 74.24%, and THI 70.49. During the night, the average temperature was 20.14°C, average relative humidity 73.85%, and THI 68.66.

The second experimental phase lasted 7 d and started with a sudden increase in the temperature up to 30°C. The mean temperature during the day was

30.65ºC, relative humidity 50.50%, and THI 79.39. During the night, the mean temperature was 28.65ºC, relative humidity 47.80%, and THI 76.77.

The third experimental phase lasted 7 d, during which the high temperature as in the second stage was maintained, but the air movement in the experimental room was increased. The average temperature during the day was 30.04°C, humidity 48.55%, THI 78.30, and air flow 3.12 m/s. During the night, the mean air temperature was 26.23°C, humidity 47.29%, and THI 73.35.

Results

Physiological response of animals to high temperature (Tables 2, 3) was examined. In stage II of the experiment, RR increased significantly ($P < 0.01$) from 56.21 ± 16.1 to 96.43 ± 41.92 breaths per minute. Also, it was a significant increase ($P < 0.05$) in HR from 90.23 ± 12.42 to 107.79 ± 28.84 beats per minute. Body temperature did not show the expected upward trend and remained constant at 39.36°C. In stage III of the experiment, a significant decrease ($P < 0.01$) in RR from 96.43 ± 41.92 to 57.60 ± 23.27 breaths per minute and a decrease in HR from 107.79 ± 28.84 to 12.17 ± 80.8 beats per minute occurred (Table 3). Blood analysis showed that white blood cell count (WBC) significantly ($P < 0.05$) decreased from 10.07 ± 2.1 to 9.12 ± 0.7 k./µL in stage II and from 9.12 ± 0.7 to 7.07 ± 1.7 k./µL in stage III. When comparing results of stage I to results of stage III, a highly significant ($P < 0.01$) difference of WBC concentration can be noted (Table 4). In stage II of the experiment, a highly significant ($P < 0.01$) increase in concentrations of calcium from 2.27 ± 0.4 to 2.67 ± 01 mmol/L, chlorine from 98.16 ± 12.5 to 109.44 ± 1.8 mmol/L, and potassium from 4.11 ± 0.7 to 4.47 ± 0.2 mmol/L were observed. When comparing stage I with stage III, a significant increase in iron concentration was observed (Table 5). In stage II, a significant ($P < 0.05$) increase in the concentration of cortisol from 5.88 ± 2.8 to 7.97 ± 1.8 ng/dL was noted. In stage III, however, there was a highly significant ($P < 0.01$) decrease in the concentration of cortisol from 7.97 ± 1.8 to 4.48 ± 2.6 ng/dL (Table 6).

Table 2. Environmental parameters during the experiment

		Temperature	Humidity	Ammonia	Hydrogen sulfide	THI	Air movement
STAGE I	day	20.95	74.24	0.87	0.14	70.49	0.26
	night	20.14	73.85	0.98	0.11	68.66	0.26
STAGE II	day	30.65	50.50	1.47	0.21	79.39	0.30
	night	28.65	47.80	1.65	0.17	76.77	0.30
STAGE III	day	30.04	48.55	2.60	0.20	78.30	3.12
	night	26.23	47.29	2.53	0.14	73.35	3.12

day (9:00-19:00), night (19:00-9:00)

Table 3. Physiological parameters during the experiment

	Stage I $_{(n = 45)}$	±	Stage II $_{(n = 45)}$	±	Stage III $_{(n = 45)}$	±
Beats/min	90.23^{a}	12.4	107.79^{Ab}	28.8	80.80^{B}	12.2
Breaths/min	56.21^{A}	16.0	96.43^{B}	41.9	57.60^{A}	23.3
Temperature (ºC)	39.36^{A}	0.4	39.36^{A}	0.3	38.97^{B}	0.4

A,B,C - $P < 0.01$
a,b,c - $P < 0.05$
„n"-number of tested samples

Table 4. Blood morphological parameters in Polish Merino sheep during the experiment

	Stage I $_{(n = 45)}$	±	Stage II $_{(n = 45)}$	±	Stage III $_{(n = 45)}$	±
WBC	10.07^{Aa}	2.1	9.12^{b}	0.7	7.07^{Bc}	1.7
RBC	9.55	1.5	8.74	1.8	8.72	1.6
HGB	6.47	0.9	5.72	1.1	5.71	1.0
HCT	0.29	0.0	0.27	0.1	0.27	0.1
PLT	558.00	193.2	594.56	145.2	472.86	116.7
MCV	30.73	r0.8	30.39	0.8	30.43	0.6
MCH	0.68^{a}	0.0	0.67	0.0	0.66^{b}	0.0
MCHC	22.13	1.0	21.85	1.1	21.56	1.0

A,B,C - $P < 0.01$
a,b,c - $P < 0.05$
„n"-number of tested samples

Table 5. Blood biochemical parameters in Polish Merino sheep during the experiment

	Stage I $_{(n = 45)}$	±	Stage II $_{(n = 45)}$	±	Stage III $_{(n = 45)}$	±
AST	73.07	38.8	86.46	51.8	102.81	74.4
Glu	3.37	0.5	3.49	0.2	3.60	0.2
K	4.11^{a}	0.7	4.47^{b}	0.2	4.51^{b}	0.2
Cl	98.19^{A}	12.5	109.44^{B}	1.8	111.41^{B}	2.2
Fe	14.80^{a}	5.1	16.85	4.5	20.06^{b}	5.7
Ca	2.27^{A}	0.4	2.67^{B}	0.1	2.64^{B}	0.1
CK	146.16	73.2	170.02	34.9	171.55	79.8

A.B.C - P < 0.01
a.b.c - P < 0.05
„n”-number of tested samples

Table 6. Level of blood cortisol in Polish Merino sheep during the experiment

	Stage I $_{(n = 45)}$	±	Stage II $_{(n = 45)}$	±	Stage III $_{(n = 45)}$	±
Cortisol	5.88^{a}	2.8	7.97^{Ab}	1.8	4.48^{B}	2.6

A.B.C - P < 0.01
a.b.c - P < 0.05
„n”-number of tested samples

Discussion

Blood analysis as well as the physiological parameters in the first part of the experiment fulfilled the accepted standard values for sheep (19). This means that the results obtained in the further part of the experiment were not affected by physiological instability or poor health status of animals.

The second stage of experiment (high temperature and low air movement) led to thermal stress in tested animals. It was indicated by increased respiratory and heart rates. Animals showed an increase in HR from 90.23 ± 12.42 in the first stage to 107.79 ± 28.84 in second stage. Similar results were presented by Mittal and Gosh (10). In their experiment, HR increased from 70.2 ± 5.85 to 102.0 ± 13.91 when animals were exposed to high temperatures. The opposite trend was observed in animals exposed to high temperatures in the study of El Sheikh *et al.* (3), who demonstrated the values of 88.0 ± 11.0 for thermally neutral conditions and 73.6 ± 9.8 for high temperature. However, in the same study, the results for tropical breeds of sheep were shown: HR in Ossimi breed was 66.7 ± 2.5 under thermally neutral conditions and 77.8 ± 3.2 in thermal stress. It is hard to find a clear tendency for this parameter. It is recognised that there is a natural increase in HR when the animal is under the influence of high temperature. At very high temperatures, HR may decrease as a result of the slowdown of animal metabolism (1). In our study RR increased to 56.21 ± 16.01 in the first stage and to 96.43 ± 41.92 in the second stage of the experiment. This result seems to be in accordance with observations of Srikandakumar *et al.* (16), who gave a value of 50 ± 2.1 for normal temperature and 128 ± 2.5 for animals exposed to high temperatures. Higher values in the mentioned studies can be explained by higher THI rates (93 ± 3.1), while in the present study it was 78.54 ± 1.47. Slightly different values where observed by El Sheikh *et al.* (3): 76.3 ± 6.3 for neutral temperature and 104.0 ± 7.2 for the thermal stress, and by Mittal and Ghosh (10): from 45.0 ± 3.39 at the morning (8:00) to 110.2 ± 16.92 at the hottest part of the day (15:00). However, Singh *et al.* (15) recorded much higher values: 98.0 ± 1.9 at 24ºC and 271.0 ± 22.6 at 40°C. Although values indicated in various studies slightly differ, they all exhibit the same upward trend. The mentioned differences probably result from a variety of sheep breeds used for the studies or the thermo-humidity conditions. Nevertheless, it is clear that all authors confirm the increase in RR at elevated temperatures as in high temperatures metabolism of animals is increased. That increases the demand for oxygen in tissues. As a result, RR (O_2 absorption) and HR (O_2 distribution) increase.

The level of cortisol in the blood increased significantly in animals exposed to high temperature, indicating the occurrence of heat stress. Psychological stress can increase concentration of cortisol in blood with a positive effect on the thermogenesis and negative on heat tolerance (11). Cortisol level significantly increases in animals exposed to high temperatures and gradually decreases during long-term exposure to these temperatures. Decrease in cortisol levels during chronic heat stress is the result of animal’s adaptation to the acute thermal conditions (14).

The body temperature in the second stage of experiment remained unchanged. Srikandakumar *et al.* (16) described an increase in body temperature from 39.5 ± 0.06 to 39.8 ± 0.008 in animals exposed to heat stress. Greater increase was observed by Singh *et al.*

(15) who presented the value of 39.8 ± 0.1 for ambient temperature of 24ºC and 41.2 ± 0.1 for ambient temperature of 40°C. El-Seikh *et al.* (3), on the other hand, observed an increase in body temperature from 40.6 ± 0.2 in the morning to 40.7 ± 0.1 at the hottest part of the day (15:00). The body temperature of sheep under thermoneutral conditions is within the range of 38.3 to 39.9°C. Changes in body temperature in this range are natural for sheep. Exceeding the temperature indicates the loss of thermo-physiological stability, which means that the animal is not able to cope with the high temperature (15). No significant difference in body temperature between the first and second stage of the experiment shows that animals were able to lose excess heat and did not lose their stability. As a result, the body temperature remained unchanged.

Blood parameters have also changed in the animals exposed to high temperatures. The level of WBC decreased after the occurrence of heat stress. Decrease of this parameter was also noted by Gomes da Silva *et al.* (4). Study by Uwayjana and Bhattacharya (18) showed an increase in WBC in animals exposed to thermal stress. The rest of indicators did not change significantly.

In the third stage of the experiment, animals showed a decrease in HR and body temperature. It can be concluded that air movement in experimental room accelerates heat exchange by convection, as hot air is removed quicker from the body surface. Evaporation is also accelerated by increased air movement. As a result, body temperature and demand for oxygen in tissues decreased. This may prove that heat stress was reduced by forced air movement. At this stage of the experiment, air movement was set at 3.12 m/s, while in the previous two stages average air movement was 0.26 and 0.30 m/s.

It is interesting to compare the results of the third stage and the first stage of the experiment. Although in the third stage the temperature was higher than in the first stage, an increased air flow was induced, causing a decrease in parameters such as HR and body temperature to values lower than at the beginning of the experiment. Cortisol level decreased from 5.88 in stage I to 4.48 in stage III, as well as HR from 90.23 in the first stage to 80.8 in the third. Body temperature that remained stable (39.36°C in the first and second stages), also decreased to 38.37°C. These results may indicate a high adaptability of the tested animals. It can be assumed that this is a result of mitigating effect of air movement and animal adaptation. It is also possible that during the experiment animals got accustomed to researchers, service personnel, and research activities, and therefore their stress decreased.

The study demonstrated that high air temperature (30°C) causes heat stress in Merino sheep. It was evidenced by changes in physiological, morphological, biochemical, and endocrine parameters in the blood. It was also proven that even small air movement (3.12 m/s) has mitigating effect on heat stress in sheep. An increase in air movement from 0.3 to 3.12 m/s resulted in faster and more efficient convective heat loss by body surface. It can be assumed that air movement should be considered as an important factor in heat stress reduction and may be used in practice. The use of natural air movement could be implemented in farm building construction. Also, it could be used in agriculture practice as farmers could use fans in farming buildings in order to create additional air movement (regular air exchange in the farm buildings is usually not sufficient to create desired air flow) that is particularly important during the summer season.

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